Send Orders for Reprints to reprints@benthamscience.ae

Mini-Reviews in Medicinal Chemistry, 2015, *15*, 000-000

Carbazole Derivatives: A Promising Scenario for Breast Cancer Treatment

Anna Caruso[1#], Domenico Iacopetta[1#], Francesco Puoci[1], Anna Rita Cappello[1], Carmela Saturnino[2,*] and Maria Stefania Sinicropi[1,*]

M.S. Sinicropi

[1]*Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, 87036 Arcavacata di Rende, (CS), Italy;* [2]*Department of Pharmaceutical and Biomedical Sciences, University of Salerno, 84084 Fisciano (SA), Italy*

C. Saturnino

Abstract: Chemotherapeutics used in cancer treatment may elicit pleiotropic effects interfering, for instance, directly on DNA metabolism or on endoplasmic organelles functions. Recently there has been a trend towards the use of molecular-targeted therapies as alternative treatments of cancer, arising from the need to overcome the onset of undesired side effects or drug-resistance. Thus, a major challenge is the design and synthesis of new agents able to interact with specific cellular components, often over-expressed or altered in cancerous cells, such as telomerase and topoisomerase or protein kinases, with reduced toxicity at effective doses. The main molecular targets for the development of new anticancer drugs include: cell surface receptors, signal transduction pathways, enzymes, gene transcription, ubiquitin-proteasome/heat shock proteins, and anti-angiogenic agents. Several natural or synthetic polycyclic molecules with carbazolic nucleus, which show attractive drug-like properties, were identified with the aim to increase their biological activities and their specificity, obtaining cytotoxic agents effective in a panel of cancer cell lines. The cytotoxic profile of these compounds has been assessed using several *in vitro* assays as, for instance, MTT, colony formation, and flow cytometry assays and some of these compounds showed an interesting profile at sub-micromolar concentrations. The usefulness of some carbazole derivatives has been demonstrated, as well, in preclinical studies.

Keywords: Anticancer drugs, alkaloids, ellipticine, breast cancer, carbazole derivatives, polycyclic compounds.

1. INTRODUCTION

DNA has been a remarkable bioreceptor for a large number of molecules, and it still remains one of the major biological targets for the design of anticancer agents [1]. Amongst them, carbazole derivatives represent an important and heterogeneous class of anticancer agents, which have been reported not only to intercalate DNA but also to inhibit telomerase and topoisomerase activity and regulate protein phosphorylation [2-4]. These alkaloids drew a growing interest over the last two decades and few representatives as, for instance, ellipticine [5,11-dimethyl-6*H*-pyrido [4,3-*b*]carbazole] (Table **1**) and rebeccamycin [1,11-dichloro-12-(4-*O*-methyl-β-D-glucopyranosyl)-12,13-dihydro-5*H*-indolo [2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione] (Table **1**), two naturally occurring carbazole alkaloids, have been routinely used for the treatment of cancer. Starting from ellipticine and from rebeccamycin many analogues, able to interact with specific cellular compartments (as nucleus, mitochondria and endoplasmic reticulum [5]), have been synthesized and tested for their antitumor activity, showing satisfactory results in several cancer types and, most importantly, in metastatic breast cancer. Among them both natural and synthetic carbazoles [6], either in a pure substituted or heterocyclic-condensed form, as pyridocarbazoles [7, 8], indolecarbazoles [9, 10], pyranocarbazoles [11], pyrrolocarbazoles [12, 13], benzocarbazoles [14], oxazinocarbazoles [15] and others have been reported.

Because of their polycyclic, planar and aromatic structure, carbazoles activity is strictly related to their ability to intercalate DNA, which remains one of the main targets for cytotoxic carbazoles. On the other hand, carbazole derivatives interfere, as well, with DNA-dependent enzymes, as topoisomerases I/II and telomerase, or with other targets such as cyclin-dependent kinases and estrogen receptors [16, 17].

This review provides the most salient facts on some already known carbazole derivatives and summarizes the progress of research striving to identify, and study in depth, more effective and selective classes of carbazole derivatives

*Address correspondence to these authors at the Department of Pharmacy, Health and Nutritional Sciences University of Calabria, Arcavacata di Rende (CS), 87036, Italy, Tel: +39 0984 493200; Fax: +39 0984 493298; E-mail: s.sinicropi@unical.it; and Department of Pharmaceutical and Biomedical Sciences, University of Salerno, 84084 Fisciano (SA), Italy, Tel: +39 089 969769; Fax: +39 089 969602; E-mail: saturnino@unisa.it

#These authors equally contributed to the work.

1389-5575/15 $58.00+.00

with reduced side effects. Some of them have entered clinical trials and have been approved for the treatment of cancer [6] and others have shown interesting and promising properties *in vitro* and *in vivo* that may be exploited as valid alternatives in breast cancer therapy.

2. BREAST CANCER: AN OVERVIEW

In spite of the significant progresses in cancer prevention, early diagnosis and treatment, in the last few decades reports on carcinoma cases have shown a drastic increase, mostly due to changes in lifestyle and environment. Today, research studies still uncover lifestyle factors or habits which may influence breast cancer risk but some studies evidenced changes in metabolic biomarkers, *e.g.* insulin and insulin-like growth factors, and that body fatness in mid- and later-life may increase the risk in postmenopausal women, although these findings are not yet consistent [18, 19]. The two most common types of cancer in females are represented by cervical and breast cancer, indeed the American Cancer Society (ACS) estimates more than 12.000 new cases of diagnosed cervical cancer in 2011, whereas breast cancer has become the most commonly diagnosed malignant tumor in women, accounting for 24% of all female cancer and being the second most lethal cancer among women. A major contribution to the rise of the mortality has been attributed to the development of resistance against chemotherapeutics, for instance to *cis*platin [20].

Many strategies have been accomplished with the hope to diminish the incidence and mortality rates, such as the reduction of hormone replacement therapy [21], a better prevention and improved early detection and treatment. Even though encouraging advances have been made in diagnosis and clinical practice, a trend towards a multifaceted therapeutic approach, which targets pathways promoting or sustaining cancer cell growth and invasion with a minimal effect on normal cells, has been recorded [22, 23].

One of the most widely used strategies in breast cancer therapy has been the induction of the programmed cell death, namely apoptosis, in tumor cells but, beside, several pathways are potential targets for cancer treatments. Amongst them, pathways involved in cell cycle regulation, angiogenesis, in maintaining the cellular redox state or even those triggered by membrane and nuclear receptors are also considered clinically useful [24-26]. Great advances have been made in the diagnosis, prevention and treatment of the estrogen receptor (ER) positive breast cancer and, most recently, several breast cancer trials targeting the human epidermal growth factor receptor 2 (HER2) have demonstrated their clinical efficacy. Nevertheless, the identification and development of effective, safe and selective therapies for the treatment of the so called triple negative breast cancers (TNBC), which lack of ER, progesterone receptor (PR), and HER2, still remains challenging.

The difficulty in adopting an adequate pharmacological therapy is enhanced by the inherent heterogeneity of tumors, most importantly TNBC tumors, but also by the resistance occurrence, pointing out the attention on the need for subtype-specific multi-targeted approaches [27]. Lastly, the expression of androgen receptor (AR) in TNBC, where it can function independently of ER and PR but may concert with other signal transduction pathways leading to an aggressive phenotype, is considered important for survival outcome and prognosis and, additionally, AR may be an useful target in patients with TNBC or hormone resistant breast cancer [28].

Another attractive and common strategy for breast cancer treatment consists in tumor cells angiogenesis inhibition, given that several pro-angiogenic factors are responsible of quiescent endothelial cells activation and migration into the tumor, with consequent metastasis development. Routinely used chemotherapeutic agents for breast cancer treatment exhibit anti-angiogenic activity [25, 29], so that drugs which hold both anti-proliferative and anti-angiogenic activities may represent a new and very useful strategy. Relevant to this point, carbazole derivatives exhibit different ranges of activities, including anti-estrogenic, anti-proliferation and anti-angiogenesis properties, which may be combined also in the same molecule with the aim to obtain a chemotherapeutic endowed of two activities which targets at different tumoral pathways (e.g. apoptosis and angiogenesis) [30].

3. CARBAZOLE DERIVATIVES: PAST, PRESENT AND FUTURE DIRECTIONS IN BREAST CANCER TREATMENT

3.1. Tricyclic Structures: Carbazole Derivatives Differently Substituted

Carbazoles, whether synthetic or naturally occurring, constitute an important class of indole-containing heterocycles [2] known for their potent antitumor activities [4, 31] but also for antibacterial, anti-inflammatory, psychotropic, and anti-histamine properties [2, 8, 32].

The precise mechanisms of their anti-neoplastic activity have not yet been fully explained, however it was suggested that the prevalent mechanisms of the antitumor, mutagenic and cytotoxic activities is based on their intercalation into DNA and inhibition of DNA-topoisomerase II activity [33].

Amongst these, carbazole sulfonamides represent a novel promising class of antimitotic agents with clinical development potential. In particular, a novel synthetic tubulin ligand with small molecular weight and a tricyclic planar structure, has been used successfully *in vitro* against MCF-7 breast cancer cells and human hepatocellular carcinoma SMMC-7721 cells, that is IG-105 (*N*-(2,6-dimethoxypyridin-3-yl)-9-methylcarbazole-3-sulfonamide) (**1**), synthesized by Wang *et al.* [34].

In a similar fashion to that of Vinca alkaloids, the main action of IG-105 (**1**) is to inhibit microtubule assembly through its high specific interaction with colchicine pocket within tubulin structure. Modeling studies suggest that the dimethoxypyridine moiety and the carbazole nucleus of IG-105 (**1**) are responsible of important interactions with hydrophobic pockets of tubulin and that, unlike colchicine, the sulfoamino group and the N atom of carbazole nucleus form new hydrogen bonds, leading to microtubules disruption and cell cycle arrest at M phase [35, 36], supported by cyclin B1 degradation abolition. It is noteworthy that the killing effect of IG-105 (**1**) is identical on MCF-7 cells expressing or not expressing Pgp, a feature

which makes IG-105 very interesting among anticancer tubulin ligands because its usefulness on drug-resistant tumor cells.

Moreover, IG-105 (**1**) exhibits impressive anticancer effects *in vivo*; indeed IG-105 (**1**) monotherapy has been shown to inhibit tumor growth in animals of a value about 93%, at nontoxic dose. IG-105 (**1**) higher doses were more effective compared to the lower ones, compatible with the theory that high-dose chemotherapy might be potentially curative for certain types of cancer. Another important feature of this compound is the low toxicity in animals, given that no damage on liver, kidney, heart, lung, and spleen after the mice received 1,000 mg/kg IG-105 were observed, providing a substantial advantage of the compound with respect to other tubulin ligands.

In general, sulfonamides constitute a useful class of drugs, displaying a variety of activities including antibacterial, anticarbonic anhydrase, diuretic, hypoglycemic, and antithyroid effectiveness [37-39]. Since the discovery of E-7010 (*N*-[2-[(4-hydroxyphenyl)amino]-3-pyridinyl]-4-methoxybenzenesulfonamide) in 1992, [40] a number of sulfonamides have been reported to be potent tubulin polymerization inhibitors and antiproliferative agents [38]. In particular, Hu *et al.* [41] have synthesized and evaluated two series of carbazole sulfonamides related to Combretastatin A4 and, among these, the 9-ethyl-*N*-(3,4,5-trimethoxyphenyl)-carbazole-3 sulfonamide (**2**) showed significant antitumor activity in two human xenograft models (MCF-7 and Bel-7402). The structure-activity relationships (SAR) revealed that the nitrogen atom of carbazole and the alkylation of N-9 are very important for its activity indeed, the replacement of carbazole with dibenzofuran lead to a loss of the activity. 3,4,5-Trimethoxyphenyl substituents on the phenyl ring yield potent activities, even though this array is not essential because the presence of 2,4-dimethoxy, 2,5-dimethoxy, 2,4,6-trimethoxy, 5-chloro-2,4-dimethoxy, and 4-chloro-2,5-dimethoxy on the phenyl ring do not reduce the activity. Lastly, the extension of molecular length of -SO_2NH- by an extra methylene group, linked to the nitrogen atom, resulted in significant loss of activity. Preliminary studies demonstrated that the compound (**2**) arrests tumor cell cycle at M-phase, inducing apoptotic cell death by increasing expression of p53 and promoting bcl-2 phosphorylation.

Different chemotherapeutic agents, routinely used in breast cancer treatment, are well known to exhibit anti-angiogenic activity [42]. Indeed, invasive breast cancer generally expresses certain angiogenic factors, including the vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) responsible of endothelial cells migration and proliferation and blood vessels formation, therefore drugs which target both angiogenesis and cancer cell growth inhibition have, for sure, a therapeutic value for cancer treatment. Recently, Liu *et al.* [30], have studied a novel synthetic carbazole derivative, namely HYL-6d (9-[(6-chloropyridin-4-yl)methyl]-9*H*-carbazole-3-carbinol) (**3**), very effective as antitumor agent against human breast cancer cell line MCF-7. In fact, HYL-6d exposure leads to cell cycle arrest, according to another synthetic analog pyrrolo[3,4-c]carbazole published in 2003 [43], and apoptotic cell death, demonstrated by morphological changes, chromatin condensation and internucleosomal DNA fragmentation studies. Most importantly, HYL-6d (**3**) treatment significantly inhibits angiogenic processes including proliferation and migration in VEGF- or bFGF-stimulated human umbilical vein endothelial cells (HUVECs) under pathological angiogenic conditions, a feature which plays a key role in breast cancer progression and metastasis formation inhibition.

The ability of some natural carbazole scaffolds to interfere with DNA metabolism is already known. S. Yoon *et al.* [44] have synthesized novel carbazole derivatives by adding epoxypropoxy or thioepoxypropoxy groups to the carbazole scaffold. Based on previous studies, they have hypothesized that these additions would increase the ability of carbazole to cause DNA damage. Indeed, MHY407 (9-methyl-2-(oxiran-2-ylmethoxy)-9*H*-carbazole) (**4**) showed a good DNA damaging ability and its efficacy relies on C-PARP production, topoisomerase II inhibition and cell cycle arrest at S phase by regulating cyclin D1, pRb, and p21 proteins. Based on a more detailed analysis at molecular levels, MHY407 (**4**) activity may be more multifaceted as one could expect, because its ability to block cell cycle at S phase, unlike the well-known topoisomerase inhibitors doxorubicin and etoposide, which cause S and G2/M phases arrest [45, 46]. The authors hypothesize that MHY407 (**4**) may have benefits if used as combination with doxorubicin, etoposide, or radiation, exploiting these features on MCF-7 and, most importantly, on triple-negative Hs578T breast cancer cells.

DNA structure interactions are also the mode of action of novel carbazolylguanidines synthesized starting from 3-aminocarbazole derivatives, as reported by Caruso *et al.*, through a simple and facile one-pot reaction [47]. The novel compounds prepared were tested for their anticancer activity against several cell lines and, in particular, the compound *N*-(6-bromo-1,4-dimethyl-9*H*-carbazol-3-yl)-*N'*-(pent-3-yl)-guanidine (**5**) showed a potent antiproliferative activity against MCF-7, HCT116, PC3, MRC5 cell lines. As well in this case, this correlates with the DNA binding properties of *N*-(1,4-dimethyl-9*H*-carbazol-3-yl)-*N'*-alkylguanidines (in particular: *N*-(1,4-dimethyl-9*H*-carbazol-3-yl)-*N'*-propylguanidine; *N*-(1,4-dimethyl-9*H*-carbazol-3-yl)-*N'*-*n*-butylguanidine; *N*-(6-bromo-1,4-dimethyl-9*H*-carbazol-3-yl)-*N'*-(pent-3-yl)-guanidine; *N*-(6-bromo-1,4-dimethyl-9*H*-carbazol-3-yl)-N'-*n*-butylguanidine; *N*-(6-bromo-1,4-dimethyl-9*H*-carbazol-3-yl)-*N'*-*tert*-butylguanidine), which have been evaluated on calf thymus (CT) DNA through two widely used methods: fluorescence and absorption spectrophotometric titration [47, 48].

Other derivatives, 5,8-dimethyl-9*H*-carbazol-3-ols [8, 16, 49] have been synthesized and tested by the means of MTT assay on MCF-7 cell line. Some of these compounds (5,8-dimethyl-9*H*-carbazol-3-ol (**6**) and 5,8,9-trimethyl-9*H*-carbazol-3-ol (**7**) showed an antiproliferative activity at submicromolar concentrations, which is encouraging for using them as potential antitumor agents. Moreover, the authors observed that at higher concentrations no dose-response inhibitory effect on MCF-7 cell viability has been recorded, suggesting that a survival pathway related to

receptor desensitized at high concentration may be involved, but it remains to be established [5].

3.2. Tetracyclic Structures: Pyridocarbazoles

Several pyridocarbazole derivatives have been reported to exhibit anti-cancer and anti-human immunodeficiency virus (HIV) activities [50, 51]. In particular, ellipticine and its regioisomeric annulated indole and carbazole derivatives with pyrido[4,3-b]carbazole framework constitute an interesting class of antitumor activity drugs [52, 53]. Many experimental studies indicated that the size, shape, and planarity of the ellipticine and derivatives are important factors for their mechanism of cytotoxicity and antitumor activity. Furthermore, it appears that positions 9 and N-2 are crucial for activity whereas the positions 1 and 11 are relatively tolerant and some variations into the scaffold of the molecule are allowed [54, 55].

For example, the 9-methoxyellipticine (**8**) shows activity against a variety of human tumor cell lines, especially against leukaemia, whereas the quaternary pyridinium salt ellipticinium acetate was developed against metastatic breast cancer [56]. Le Pecq *et al*. reported that the presence at position 9 of an electrophilic substituent would permit a direct interaction, possibly through a hydrogen bond, with the negatively charged oxygen of the DNA phosphate group [57].

Apart from their utility for several disease targets, pyrido[2,3-a]carbazole derivatives have shown an enormous synthetic value in the preparation of various bio-active molecules. Attracted by the varied biological activities of pyridocarbazole derivatives, M. Saravanabhavan *et al.* have used an alternative green reaction (microwave) for the synthesis of pyrido carbazole derivatives [58].

Preclinical studies and clinical trials have shown that ellipticine and some derivatives, namely 9-hydroxyellipticine (**9**), 9-hydroxyl-N^2-methyl-ellipticinum (Elliptinium) (**10**), 9-chloro-N^2-methylellipticinium (**11**), 9-methoxy-N^2-methylellipticinium (**12**), and 9-dimethyl-amino-ethoxy-ellipticine (**13**), obtained by various synthetic methods [59-61], effectively induce growth arrest and cell death in breast adenocarcinoma [5, 62-65] and exhibit low toxic effects.

Ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-b]carbazole) (**14**) is one of the simplest plant alkaloids, first isolated from evergreen tree leaves of the Ochrosia elliptica, belonging to the Apocyanaceae family [66]. Several studies have highlighted the plethora of its biological effects, and amongst them, ellipticine and its first synthetic derivatives, elliptinium (**10**) and elliptinium acetate (Celiptium) (**15**), have been showed to exhibit antitumor activity and even employed in treatment of metastatic breast cancer [56, 62, 67, 68]. The principal mechanism of its cytotoxicity is related to the inhibition of topoisomerase II-β, however ellipticine may bind to proteins [69], induce free radicals generation [70], inhibit cytochrome P4501A1 [71], uncouple oxidative phosphorylation [72], activate the transcription function of mutant p53 [73] and induce endoplasmic reticulum stress [5].

The formation of ellipticine-DNA adducts ultimately triggers cell death signaling which involves p53-dependent pathway, G2/M cell cycle arrest, cyclinB1 and Cdc2 expression regulation (as well as phosphorylation of Cdc2), Fas/Fas ligand system activation, and the mitochondrial pathway of apoptosis [64, 74]. Although the exact molecular mechanism responsible for the cytotoxicity has not yet been explained, the ellipticine prevalent mechanisms consist in DNA intercalation and DNA topoisomerase II activity inhibition [75]. Indeed, the size and shape of the Ellipticine chromophore is closely similar to those of a purine–pyrimidine complementary base pair, necessary for its intercalation in double-stranded DNA. Moreover, the polycyclic aromatic character of the molecule (**14**) may interact with suitable hydrophobic regions in DNA and, at the same time, ellipticine methyl groups and thymine bases interactions occurring at the intercalation site are, likely, important for the ellipticine orientation [76]. The ellipticine action as inhibitor of topoisomerase II has been extensively studied, in fact ellipticine (**14**) stimulates topoisomerase II-mediated DNA breakage, probably through the formation of a ternary complex between topoisomerase II, DNA and drug [77]. Additionally, Froelich-Ammon and coworkers postulated that ellipticine (**14**) associates firstly with either DNA or the enzyme, without requiring the presence of a preformed topoisomerase II–DNA complex, and that DNA breakage is enhanced by a rise in the cleavage forward rate [78]. In this view, the ellipticine anticancer activity seems to be based on mechanisms of nonspecific drug actions, so that the ellipticine specificity should be ascribed to other mechanisms not yet deeply evaluated.

The ability of ellipticine (**14**) to interact with DNA or proteins has been investigated under different points of view, using several methods. For instance, interactions with double-stranded or even single-stranded oligonucleotides have been evaluated by electrochemical methods, showing time and dose-dependent behavior [79], or through field emission scanning electron microscopy (FE-SEM), used to assess direct morphological patterns of the protein–DNA self-assembled aggregates or complexes [80]. Another study reported a novel method based on capillary electrophoresis (CE) with laser-induced fluorescence (CE-LIF) detection which has been exploited for the determination of ellipticine (**14**) and for monitoring the interactions between ellipticine and DNA *in vitro* [81].

It is noteworthy that the chemical modifications due to metabolism processes may affect the biological activities. Indeed, Stiborova *et al.* have investigated the ellipticine metabolism and showed that several mammalian cytochrome P450 (CYP) enzymes oxidize ellipticine (**14**) into several metabolite forms. CYP1 enzymes primarily oxidize ellipticine (**14**) to form 9-hydroxy- and 7-hydroxyellipticine, which represent detoxification products whereas CYP3A4 converts ellipticine (**14**) into 12-hydroxy and 13-hydroxyellipticine which are more active forms, able to target DNA [82-84]. These findings are strengthened by the cross-resistance to ellipticine occurring in Adriamycin resistant (Adr^R) MCF-7, which would be explained by a decrease in the CYP1A1-dependent activation of ellipticine, and by the higher sensitivity to ellipticine in MCF-7 cells

expressing high levels of CYP1A [85, 86] which increases ellipticine-DNA adduct formation.

The main reason for the interest in ellipticine (**14**), and its derivatives, for clinical purposes resides in their high efficiencies against several types of cancer and their lack of hematologic and hepatic toxicity, although most frequent adverse effects consist of digestive troubles, hypertension, muscular cramp, mouth dryness, and its mutagenicity risk should be evaluated in detail. Besides, the efficacy of ellipticine derivatives as potential therapeutic agents has been problematic because of their poor aqueous solubility, so that in order to overcome this limitation researchers addressed to the formation of salts, for instance by quaternisation at N-2, as a modification leading to higher aqueous solubility and increased anti-tumor efficacy. This strategy has been revealed to be effective, indeed chemical modifications at N-2 allowed to solve not only the solubility issues but, at the same time, favor a better interaction with the DNA topoisomerase ternary complex. Even though the possibilities to chemically modify the N-2 position to obtain modified properties are very large, the only clinically successful salt, to date, is represented by the 9-hydroxy-*N*-methylellipticinium acetate (Celiptium) (**15**), used for treatment of metastatic breast cancer [87, 88].

However, the antitumor activity of ellipticine is due also to the ability in binding, with a very high affinity, to telomeric DNA sequence, H24, blocking the telomerase activity and preventing the growth and survival of MDA-MB-231 breast cancer cells [89]. Additionally, the induction of apoptosis in MDA-MB-231 breast cancer cells mediated by an ellipticine derivative, 6-propanamine-ellipticine (6-PA-ELL) (**16**), is preceded by an earlier event, that is the endoplasmic reticulum stress; in fact 6-PA-ELL (**16**) is able to up-regulate the expression of chaperones and to interfere with protein folding, provoking the accumulation of misfolded proteins in endoplasmic reticulum [5].

The water soluble derivative of ellipticine, 9-hydroxyellipticine (9HE) (**9**), is capable not only to intercalate DNA with a higher affinity but, as well, targets topoisomerase II [90], telomerase [91], cytochrome P450 [92], and p53 [93]. Short time 9HE treatment leads to a decrease of p53 phosphorylation, while prolonged treatment blocks cell cycle at G0/G1 phase and triggers p53-mediated apoptosis in ER-negative SK-BR-3 breast cancer cells [94]. However, more recently it has been shown that 9HE is an efficient and selective inhibitor of eukaryotic polymerase-I (Pol-I) transcription *in vitro* and this inhibition occurs independently of p53, ataxia telangiectasia mutated/ATM and Rad3-related (ATM/ATR) kinases, or topoisomerase II [95] in MCF-7 cells, as well as in other cell lines.

To widen even more the knowledge of the effects elicited by ellipticine (**14**), another interesting study has been conducted on breast cancer stem cells (BCSCs), which represent one of the major obstacles in cancer treatment, very difficult to eradicate and responsible of cancer relapse phenomena. In this study ellipticine (**14**) has been demonstrated to bind and efficiently inhibit the expression of aldehyde dehydrogenase 1 class A1 (ALDH1A1) in two models of breast cancer cells, MCF-7 and SUM159, leading to a reduction in proliferation and self-renewal ability of BCSCs, which can be potentiated through the paclitaxel co-administration [96].

More recently, interesting other series of pyrido[2,3-a]carbazoles have been prepared by catalyzed microwave reaction of 1-chloro-2-formyl carbazole with ethanolamine [58]. The DNA intercalative binding and cleavage properties of these compounds have been assessed and they also showed weak to moderate capacity for free radical scavenging. Cytotoxicity studies, carried out using the MTT assay method, on MCF-7 breast cancer cell line demonstrated a good activity for some compounds (8-chloro-2,3-dihydro-1*H*,11*H*-pyrido[2,3-a]carbazol-4-one (**17**) and 8-bromo-2,3-dihydro-1*H*,11*H*-pyrido[2,3-a]carbazol-4-one (**18**) showed IC_{50} values of 44.26 μM and 47.15 μM, respectively), even though they do not reach the potential of *cis*platin. Their antigrowth properties have been referred to the electron withdrawing groups that enhance the anticancer activity and the chloro- and bromo- derivatives showed a better cytotoxic activity than the methyl- and methoxy-analogs.

3.3. Polycyclic Compounds: Indole-Carbazoles and Other Carbazolic Derivatives

Amongst pentacyclic derivatives, a family endowed with interesting biological effects on cell migration is represented by indole-carbazoles. An important mechanism involved in tumor growth, migration, invasion and metastasis [97] is represented by epithelial-to-mesenchymal transitions (EMT), supported by matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptidases, which degrade and reconstruct extracellular matrix (ECM) [98, 99]. The MMP overexpression is widespread in cancer, playing a critical role in breast cancer invasion, metastasis and tumor angiogenesis and progression through multiple mechanisms [100]. Regarding these aspects, Ho *et al.* [101] have reported that the treatment with indolo[3,2-b] carbazole (ICZ) (**19**) significantly inhibits the migration of breast cancer cells by suppression of the EMT process and by reduction of MMP-2 and -9 activity through repression of focal adhesion kinase (FAK) mRNA expression, responsible of several biological effects, such as cell adhesion, survival, differentiation, migration, invasion and angiogenesis [102, 103]. These outcomes indicate ICZ (**19**) as potential candidate for the treatment of metastatic breast cancers.

Several and important biochemical activities are also exerted by different indolocarbazole (INDO) derivatives, reported by Chen *et al*. [104], which include induction of TOP1-mediated DNA damage (T1DD) in mammalian cells [105-107]. The interest in this class of compounds (for example: rebeccamycin (**20**), and its analogs (**21**) and (**22**) arises from their radiosensitization (RS) features in mammalian cells, indeed they are active at low concentrations with little or no cytotoxicity and exhibit higher potency with respect to that of camptothecin. In other words, these INDO derivatives are able to enhance tumor control selectively at the irradiated site(s), causing no significant systemic untoward effects. The suppression of the repair capacity for "sublethal damage" of the irradiated cells

effects may be explained through two possible mechanisms: one is based on interference with cellular machinery that is responsible for repairing sub-lethal DNA damage caused by ionizing radiation, the other one is represented by the efficiency in eliminating the S-phase population of the cell cycle, relatively more resistant to radiation, contributing to the "shoulder" of the radiation survival curve [108]. Some INDO derivatives are capable of trapping TOP1 cleavable complexes, inducing RS in mammalian cells at relatively non-cytotoxic concentrations, even though studies regarding the critical structural and biochemical determinants for the induction of RS by these INDO derivatives still lack [104].

It is important to note that the NSC 655649 (**23**), a glycosyl-dichloro-indolocarbazole derivative of rebeccamycin, has been proposed as novel cytotoxic agent targeting topoisomerase and DNA repair process and has been employed in the treatment of refractory breast cancer using two different treatment schedules [109]. This rebeccamycin analog has been well tolerated in patients, with modest and manageable hematological and non-hematological toxicity. Furthermore, (**23**) has a clinical activity in advanced breast cancer, yielding a 12% response rate in this study, and achieving responses in patients with prior anthracycline-based therapy, even though not necessarily anthracycline-resistance has occurred. The compound (**23**) has been studied also as treatment in other solid tumors and the toxicity profile reported in randomized phase II study of breast cancer patients well correlates with that recorded by treatment studies in other tumor types. This study recruited patients with refractory breast cancer, including them treated with 1 or 2 prior chemotherapy regimens, or who recurred within 12 months of adjuvant chemotherapy treatment, and mostly received anthracycline and taxane-based chemotherapy. The data reveal that the drug is active in advanced breast cancer and in anthracycline-treated patients, yielding response activity on an order of magnitude consistent with other widely used chemotherapeutic and biological agents for metastatic breast cancer [109].

Other bridged rebeccamycin derivatives have been synthesized by Marminon *et al.* [110] and are very interesting because some of them exhibit a dual mode DNA targeting, either directly or via topoisomerase I inhibiting and acting on the checkpoint kinase 1 (Chk1). In contrast with other Chk1 inhibitors, which need to be used in combination with a DNA damaging agent, these compounds present the advantage to possess two complementary activities in one compound. The median inhibition concentration (IC_{50}) values toward Chk1 of some of these compounds, either bridged or not, were in the nanomolar range. However, the cationic derivatives seem to exhibit the strongest interaction with DNA but at the expense of topoisomerase I inhibition, because authors noticed a loss in their capacity to stimulate the topoisomerase I-mediated DNA cleavage. Nonbridged compounds (**24**) (dechlorinated rebeccamycin), (**25)** (dihydroxy disubstituted) and (**26)** (bearing an exocyclic methylene group on the sugar ring) were capable to bind DNA and to inhibit topoisomerase I and Chk1. In the aza series studied by Marminon *et al.* [110] the compound (**27)**, in which the sugar moiety is linked to the indole nitrogen, inhibits Chk1 significantly and can also induce DNA lesions by intercalation and topoisomerase I-mediated DNA single strand breaks, with a selective cytotoxicity [111]. The DNA-binding properties depend on the position of the sugar unit, indeed compounds with the sugar linked to the indole exhibit a high affinity for DNA whereas, when the sugar is attached to the azaindole DNA binding properties are abolished [111].

Fracasso *et al.*, in 2011 [112], examined the therapeutic potential of 7-hydroxystaurosporine (UCN-01) (**28**), the first identified Chk1 inhibitor that exhibits synergistic activity with DNA-damaging agents in preclinical studies. They also conducted a phase I study to determine the maximum tolerated dose (MTD), dose-limiting toxicity (DLT), pharmacokinetic, and pharmacodynamic effects of UCN-01 (**28**), in combination with irinotecan, in patients with resistant solid tumors. Two partial responses were observed in women with TBNC. Although the clinical activity was unimpressive, probably because of the pharmacokinetic property of UCN-01 (**28**), the authors developed effective pharmacodynamic markers to evaluate target inhibition for this class of agents, indicating that Chk1 inhibition may enhance chemotherapy-induced apoptosis in TNBC p53 mutant tumors, often associated with a particularly poor survival [112, 113].

Kinase inhibition is, as well, a feature shared by the compound NPCD (naphtho [2, 1-α]pyrrolo[3, 4-c]carbazole-5,7 (6*H*, 12*H*)-dione) (**29**) [114], but with a different target, namely the cyclin-dependent kinase 4 **(**CDK4), which inhibition causes long-lasting growth arrest at G1 phase and cell death of breast cancer cell lines (such as, MCF-7, MB 231, MCF15, T47D and GI101Ap). Decreased phosphorylation of retinoblastoma (Rb) protein by D1-CDK4/6 and decreased cyclin-dependent kinase inhibitor 1B ($p27^{kip1}$) protein level may be part of the underlying mechanism.

Edotecarin (J-107088) (**30**) is a synthetic derivative [115] that inhibits the enzyme topoisomerase I through stabilization of the DNA-enzyme complex and enhanced single-strand DNA cleavage, resulting in inhibition of DNA replication and decreased tumor cell proliferation. The antitumor effects of edotecarin (**30**) as a single agent and in combination with docetaxel or capecitabine have been evaluated [116]. As a single agent, edotecarin (**30**) showed potent cytostatic activity *in vitro* in a panel of 13 breast cancer cell lines (BT20, BT474, BT549, HS578, MCF-7, MDA-MB-134, MDA-MB-231, MDA-MB-361, MDA-MB-435, MDA-MB-453, MDA-MB-468 SKBR-3, T47D); seven cell lines were highly sensitivity, whereas BT20 and BT474 cell lines were found to be resistant, probably due to overexpression of the ATP-binding cassette transporter BCRP/MXR/ABCP. In fact it has been reported that the BCRP mRNA expression level in BT20 cells is 22-fold higher than in T47D cells and six-fold higher than in SKBR-3 cells [117]. Comparisons of edotecarin and irinotecan were performed on two ERα negative breast xenograft models, SKBR-3 [118] and MX-1 [119], but the antitumor activity of both drugs as single treatment showed no differences. In particular, weekly treatments of edotecarin, at a dose of 150 mg/kg, showed significant antitumor activity against the SKBR-3 model, with no major toxicity, and cured all the

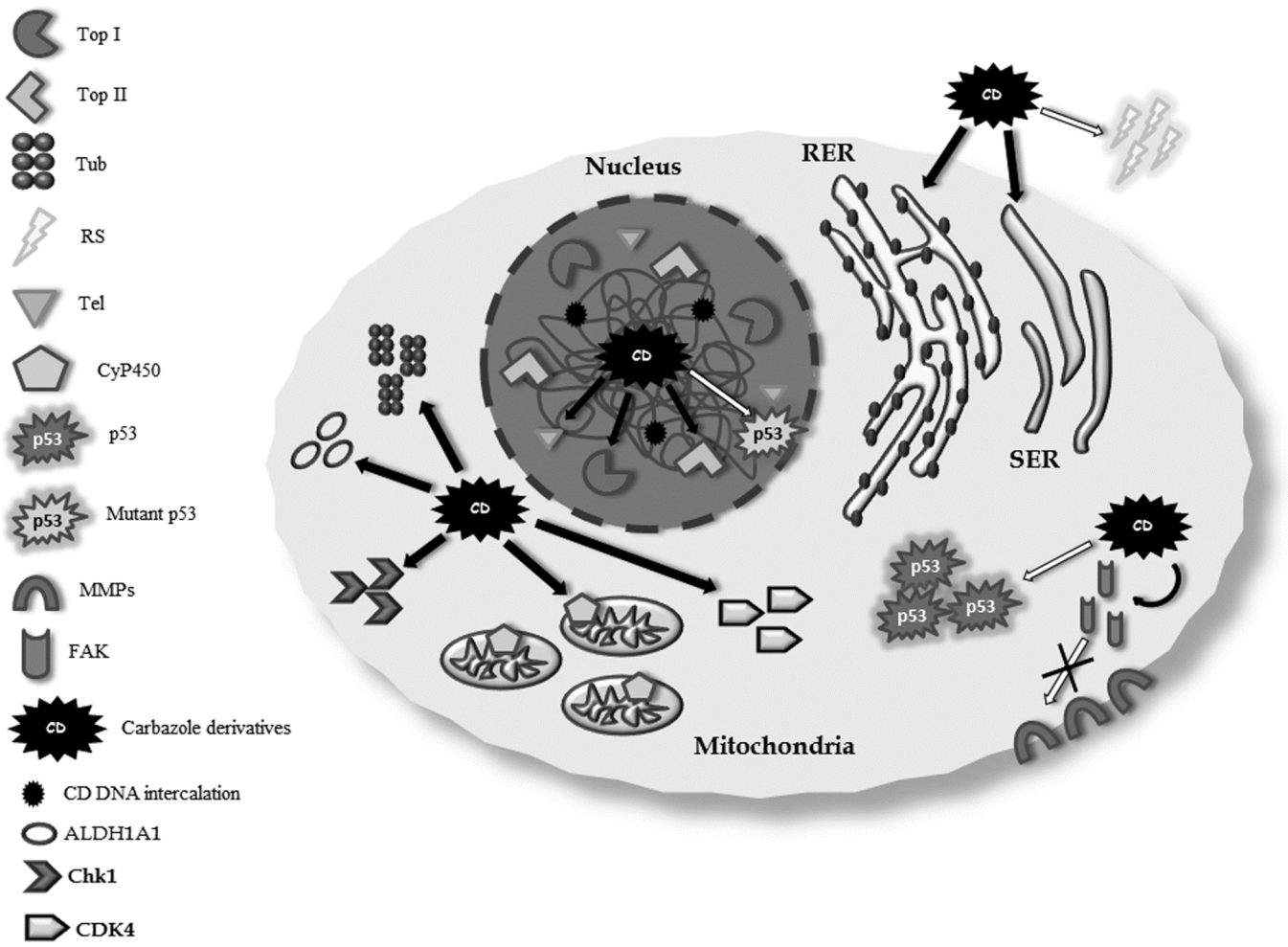


Fig. (1). A cartooned model depicting the major targets on which carbazole derivatives carry on their actions in breast cancer cells. Black arrows indicate inhibition/damage, white arrows indicate activation. RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum; Top I, topoisomerase I; Top II, topoisomerase II; Tub, tubulin; RS, radiosensitization; Tel, telomerase; CyP450, cytocrome P450; p53, protein 53, MMPs, Matrix metalloproteinases; FAK, Focal adhesion kinase; CD, carbazole derivatives; ALDH1A1, aldehyde dehydrogenase 1 class A1; Chk1, checkpoint kinase 1; CDK4, cyclin-dependent kinase 4.

animals in the MX-1 model. Treatments twice a week for two consecutive weeks produced a 75% tumor growth inhibition [120]. Then, these studies indicate that edotecarin (**30**) exhibits potent antitumor activity in human xenograft breast cancer models and is also able to enhance the effects of docetaxel and capecitabine in human breast cancer xenografts. These features make edotecarin (**30**), either as monotherapy or in combination with other chemotherapeutics, a potentially effective therapeutic option for patients with breast cancer, independent of their estrogen receptor status.

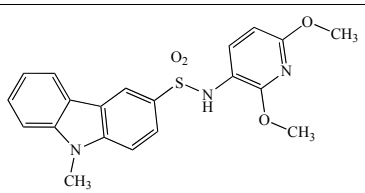
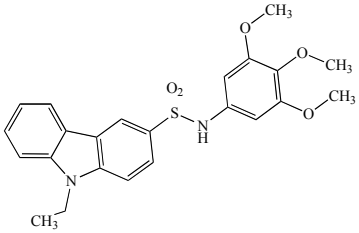
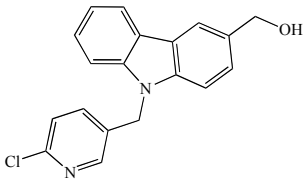
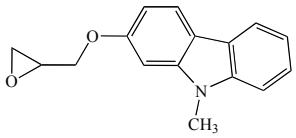
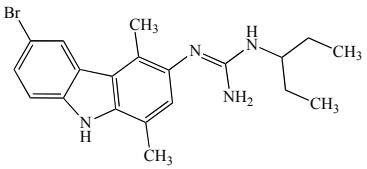
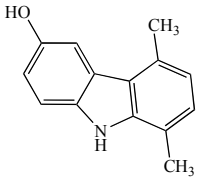
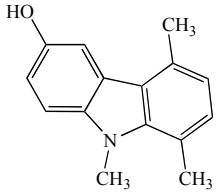
Nakamura *et al.* [121], have examined also the antitumor activity of ER-37328 (12,13-dihydro-5-[2 (dimethylamino) ethyl]-4*H*-benzo[*c*]pyrimido[5,6,1-*jk*]carbazole-4,6,10 (5*H*,11*H*)-trione hydrochloride) (**31**) *in vitro,* on human breast cancer MX-1 cells, and *in vivo* on MX-1 xenograft mice model, in comparison with etoposide or irinotecan (topo-I poison) and *cis*platin (alkylating agent), respectively. They evidenced a better antigrowth activity compared to etoposide on MX-1 cells, but a less potent effect than irinotecan and *cis*platin against MX-1 tumor xenograft. However, ER-37328 (**31**) activity on other xenograft cancer models has been revealed to be very good, so that the *in vitro* strong cell killing activity and the *in vivo* antitumor spectrum, different from irinotecan and *cis*platin, and regression activity may promote its use in clinical treatment of malignant solid tumors.

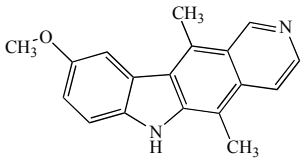
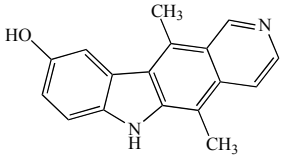
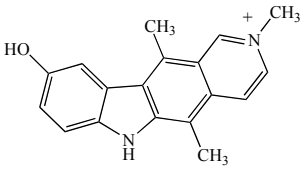
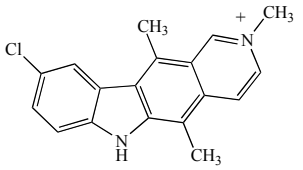
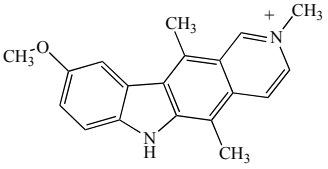
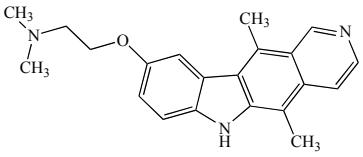
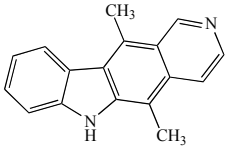
4. CONCLUSIONS

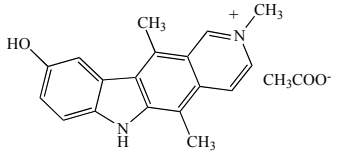
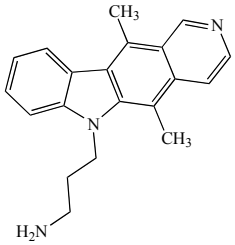
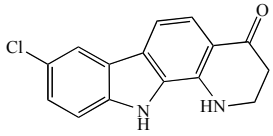
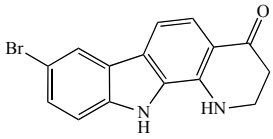
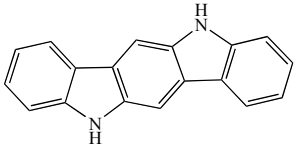
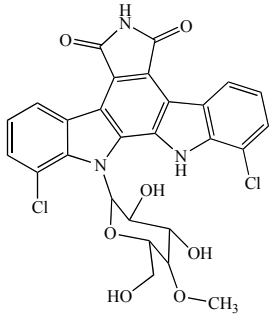
Therapies based on the induction of apoptosis have been reported to be one of the most powerful clinical strategy in cancer treatment. Great efforts to design and synthesize carbazole derivatives for cancer research have been made over the last years and the main targets on which they act have been schematically summarized in (Fig. **1**).

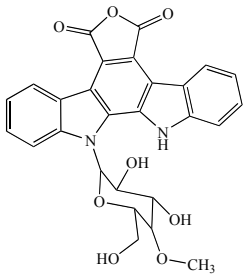
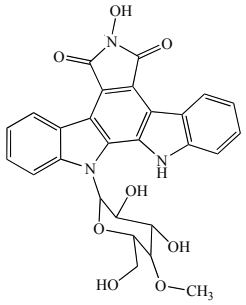
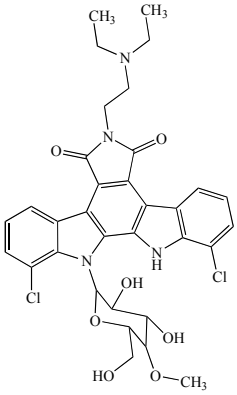
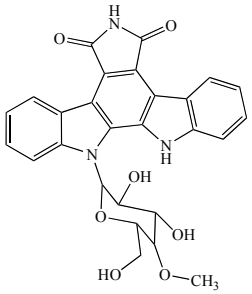
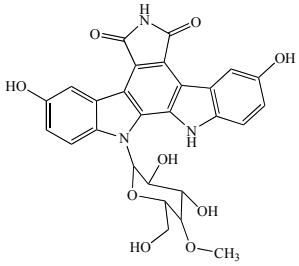
Many of them have been successfully effective in reducing cancer cell growth *in vitro* and *in vivo*, and promising compounds with high potency have also entered clinical trials. In the near future, research of new analogs

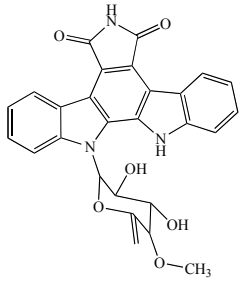
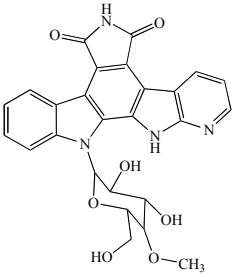
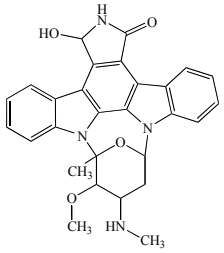
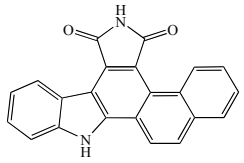
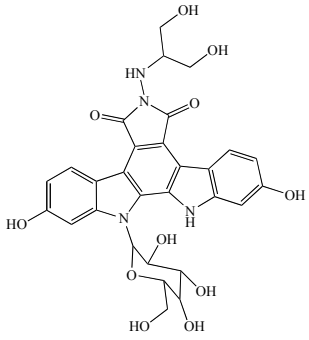
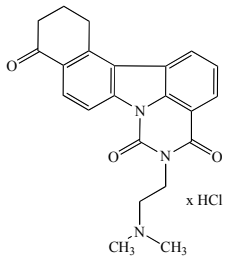
Table 1. Carbazole derivatives herein discussed and corresponding references.

Tricyclic structures: carbazole derivatives differently substituted	
Compounds	**References**
 IG-105 **1**	Wang *et al.* [34]
 **2**	Hu *et al.* [41]
 HYL-6d **3**	Liu *et al.* [30]
 MHY407 **4**	S. Yoon *et al.* [44]
 **5**	Caruso *et al.* [47]
 **6**	Caruso *et al.* [16] Parisi *et al.* [49]
 **7**	Caruso *et al.* [16]

Tetracyclic structures: pyridocarbazoles	
 9-Methoxyellipticine **8**	Svoboda *et al.* [50]
 9-Hydroxyellipticine **9**	Harding *et al* [53] Renault *et al.* [90] Sato *et al.* [91] Lesca *et al.* [92] Ohashi *et al.* [93] Sugikawa *et al.* [94] Andrews *et al.* [95]
 9-hydroxyl-N^2-methyl-ellipticinium (Elliptinium) **10**	Rouesse *et al.* [62] Treat *et al.* [67]
 9-Chloro-N^2-methylellipticinium **11**	Arguello *et al.* [65]
 9-Methoxy-N^2-methylellipticinium **12**	Andrews *et al.* [95]
 9-Dimethyl-amino-ethoxy-ellipticine **13**	Hagg *et al.* [5]
 Ellipticine **14**	Hagg *et al.* [5] Stiborova *et al.* [33] Moody *et al.* [63] Kouadio *et al.* [122] Kuo *et al.* [64, 74] Dodin *et al.* [69] Lesca *et al.* [71, 92] Schwaller *et al.* [72] Peng *et al.* [73] Monnot *et al.* [75] Singh *et al.* [76] Fosse *et al.* [77] Froelich-Ammon *et al.* [78]

	Sengupta *et al.* [80] Tmejova *et al.* [79] Ryvolova *et al.* [81] Stiborova *et al.* [82-84] Chang *et al.* [85] Ivy *et al.* [86] Ghosh *et al.* [89] Andrews *et al.* [95] Pandrangi *et al.* [96]
 Celiptium **15**	Rouesse *et al*. [56] Buzdar *et al*. [68] Deane *et al*. [87] Miller *et al*. [88]
 6-PA-ELL **16**	Hagg *et al*. [5]
 **17**	Saravanabhavan *et al*. [58]
 **18**	Saravanabhavan *et al*. [58]
Polycyclic compounds and other	***carbazole derivatives***
 ICZ **19**	Ho *et al.* [101]
 Rebeccamycin **20**	Marminon *et al.* [110]

 **21**	Chen *et al.* [104],
 **22**	Chen *et al.* [104],
 NSC 655649 **23**	Burstein *et al.* [109]
 **24**	Marminon *et al.* [110]
 **25**	Marminon *et al.* [110]

 **26**	Marminon *et al.* [110]
 **27**	Marminon *et al.* [110] Marminon *et al.* [111]
 UCN-01 **28**	Fracasso *et al.* [112] Ma *et al.* [113]
 NPCD **29**	Sun *et al.* [114]
 Edotecarin **30**	Ohkubo *et al.* [115] Ciomei *et al.* [116] Arakawa *et al.* [120]
 ER-37328 **31**	Nakamura *et al.* [121]

may pay more attention to molecules endowed of different activities, with increased selectivity and affinity and less side effects. Moreover, the design and synthesis of new *lead compounds* may help to fully explore the signaling pathways involved in cancer growth and progression.

LIST OF ABBREVIATIONS

Adr^R	=	Adriamycin resistant
ALDH1A1	=	Aldehyde dehydrogenase 1 class A1
AR	=	Androgen receptor
ATM/ATR	=	Ataxia telangiectasia mutated/ATM and Rad3-related
Bcl-2	=	B-cell lymphoma gene 2
BCRP/MXR/ABCP	=	ATP-binding cassette transporter
BCSCs	=	Breast cancer stem cells
bFGF	=	Basic fibroblast growth factor
Cdc2	=	Cell-division cycle 2
CDK4	=	cyclin-dependent kinase 4
CE	=	Capillary electrophoresis
Celiptium	=	9-hydroxy-N-methylellipticinium acetate
Chk1	=	Checkpoint kinase 1
C-PARP	=	Cleaved poly(ADP-ribose) polymerase
DLT	=	dose-limiting toxicity
DNA	=	Deoxyribonucleic acid
ECM	=	Extracellular matrix
EMT	=	Epithelial-to-mesenchymal transitions
ER	=	Estrogen receptor
FAK	=	Focal adhesion kinase
FE-SEM	=	Field emission scanning electron microscopy
HER2	=	Epidermal growth factor receptor 2
9HE	=	9-hydroxyellipticine
HIV	=	Human immunodeficiency virus
HUVECs	=	Human umbilical vein endothelial cells
IC_{50}	=	Median inhibition concentration
ICZ	=	indole[3,2-b] carbazole
INDO	=	indolocarbazole
LIF	=	Laser-induced fluorescence
MTT	=	(3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)

M	=	Mitotic
MMPs	=	Matrix metalloproteinases
mRNA	=	Messenger ribonucleic acid
MTD	=	Maximum tolerated dose
NPCD	=	(naphtho [2, 1-α] pyrrolo [3, 4-c] carbazole-5, 7 (6H, 12H)-dione)
p21	=	Protein 21
$p27^{kip1}$	=	Cyclin-dependent kinase inhibitor 1B
p53	=	Protein 53
Pgp	=	Permeability glycoprotein
Pol-I	=	Polymerase-I
PR	=	Progesterone receptor;
pRb	=	Retinoblastoma protein
6-PA-ELL	=	6-propanamine-ellipticine
RS	=	Radiosensitization
T1DD	=	TOP1-mediated DNA damage
TBNC	=	Triple negative breast cancers;
Top II	=	topoisomerase II
UCN-01	=	7-hydroxystaurosporine
VEGF	=	Vascular endothelial growth factor

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by Commissione Europea, Fondo Sociale Europeo (FSE 2007/2013-PROGRAMMA ARUE) and Regione Calabria to Domenico Iacopetta.

REFERENCES

[1] Ferri, K.F.; Kroemer, G., Organelle-specific initiation of cell death pathways. *Nat. Cell Biol.,* **2001**, *3*, (11), E255-263.

[2] Caruso, A.; Lancelot, J.-C.; El-Kashef, H.; Sinicropi, M.S.; Legay, R.; Lesnard, A.; Rault, S., A rapid and versatile synthesis of novel pyrimido[5,4-b]carbazoles. *Tetrahedron,* **2009**, *65*, (50), 10400–10405.

[3] Caruso, A.; Voisin-Chiret, S.A.; Lancelot, J.-C.; Sinicropi, M.S.; Garofalo, A.; Rault, S., Novel and efficient synthesis of 5,8-Ddimethyl-9Hcarbazol-3-ol via a hydroxydeboronation reaction. *Heterocycles,* **2007**, *71*, (10), 2203 - 2210.

[4] Caruso, A.; Voisin-Chiret, A.S.; Lancelot, J.C.; Sinicropi, M.S.; Garofalo, A.; Rault, S., Efficient and simple synthesis of 6-aryl-1,4-dimethyl-9H-carbazoles. *Molecules,* **2008**, *13*, (6), 1312-1320.

[5] Hagg, M.; Berndtsson, M.; Mandic, A.; Zhou, R.; Shoshan, M.C.; Linder, S., Induction of endoplasmic reticulum stress by ellipticine plant alkaloids. *Mol. Cancer Ther.,* **2004**, *3*, (4), 489-497.

[6] Asche, C.; Demeunynck, M., Antitumor carbazoles. *Anticancer Agents Med. Chem.,* **2007**, *7*, (2), 247-267.

[7] Ferlin, M.G.; Marzano, C.; Gandin, V.; Dall'Acqua, S.; Dalla Via, L., DNA binding ellipticine analogues: synthesis, biological evaluation, and structure-activity relationships. *Chemmedchem,* **2009**, *4*, (3), 363-377.

[8] Jasztold-Howorko, R.; Tylinska, B.; Biadun, B.; Gebarowski, T.; Gasiorowski, K., New pyridocarbazole derivatives. Synthesis and their *in vitro* anticancer activity. *Acta Pol. Pharm.,* **2013**, *70*, (5), 823-832.

[9] Sunami, S.; Nishimura, T.; Nishimura, I.; Ito, S.; Arakawa, H.; Ohkubo, M., Synthesis and biological activities of topoisomerase I inhibitors, 6-arylmethylamino analogues of edotecarin. *J. Med. Chem.,* **2009**, *52*, (10), 3225-3237.

[10] Conchon, E.; Anizon, F.; Aboab, B.; Golsteyn, R.M.; Leonce, S.; Pfeiffer, B.; Prudhomme, M., Synthesis, *in vitro* antiproliferative activities, and Chk1 inhibitory properties of pyrrolo[3,4-a]carbazole-1,3-diones, pyrrolo[3,4-c]carbazole-1,3-diones, and 2-aminopyridazino[3,4-a]pyrrolo[3,4-c]carbazole-1,3,4,7-tetraone. *Eur J Med Chem,* **2008**, *43*, (2), 282-292.

[11] Ito, C.; Itoigawa, M.; Nakao, K.; Murata, T.; Tsuboi, M.; Kaneda, N.; Furukawa, H., Induction of apoptosis by carbazole alkaloids isolated from Murraya koenigii. *Phytomedicine,* **2006**, *13*, (5), 359-365.

[12] Akue-Gedu, R.; Rossignol, E.; Azzaro, S.; Knapp, S.; Filippakopoulos, P.; Bullock, A.N.; Bain, J.; Cohen, P.; Prudhomme, M.; Anizon, F.; Moreau, P., Synthesis, kinase inhibitory potencies, and *in vitro* antiproliferative evaluation of new Pim kinase inhibitors. *J. Med. Chem.,* **2009**, *52*, (20), 6369-6381.

[13] Lemster, T.; Pindur, U.; Lenglet, G.; Depauw, S.; Dassi, C.; David-Cordonnier, M.H., Photochemical electrocyclisation of 3-vinylindoles to pyrido[2,3-a]-, pyrido[4,3-a]- and thieno[2,3-a]-carbazoles: design, synthesis, DNA binding and antitumor cell cytotoxicity. *Eur J Med Chem,* **2009**, *44*, (8), 3235-3252.

[14] Alper, P.B.; Marsilje, T.H.; Mutnick, D.; Lu, W.; Chatterjee, A.; Roberts, M.J.; He, Y.; Karanewsky, D.S.; Chow, D.; Lao, J.; Gerken, A.; Tuntland, T.; Liu, B.; Chang, J.; Gordon, P.; Seidel, H.M.; Tian, S.S., Discovery and biological evaluation of benzo[a]carbazole-based small molecule agonists of the thrombopoietin (Tpo) receptor. *Bioorg. Med. Chem. Lett.,* **2008**, *18*, (19), 5255-5258.

[15] Issa, S.; Walchshofer, N.; Kassab, I.; Termoss, H.; Chamat, S.; Geahchan, A.; Bouaziz, Z., Synthesis and antiproliferative activity of oxazinocarbazole and N,N-bis(carbazolylmethyl)amine derivatives. *Eur J Med Chem,* **2010**, *45*, (6), 2567-2577.

[16] Caruso, A.; Chimento, A.; El-Kashef, H.; Lancelot, J.C.; Panno, A.; Pezzi, V.; Saturnino, C.; Sinicropi, M.S.; Sirianni, R.; Rault, S., Antiproliferative activity of some 1,4-dimethylcarbazoles on cells that express estrogen receptors: part I. *J. Enzyme Inhib. Med. Chem.,* **2012**, *27*, (4), 609-613.

[17] Cresteil, T.; Rault, S.; Aubert, G.; Lancelot, J.-C.; Caruso, A.; Lesnard, A. 2012.

[18] Kim, J.; Choi, W.J.; Jeong, S.H., The effects of physical activity on breast cancer survivors after diagnosis. *J Cancer Prev,* **2013**, *18*, (3), 193-200.

[19] Emaus, M.J.; van Gils, C.H.; Bakker, M.F.; Bisschop, C.N.; Monninkhof, E.M.; Bueno-de-Mesquita, H.B.; Travier, N.; Berentzen, T.L.; Overvad, K.; Tjonneland, A.; Romieu, I.; Rinaldi, S.; Chajes, V.; Gunter, M.J.; Clavel-Chapelon, F.; Fagherazzi, G.; Mesrine, S.; Chang-Claude, J.; Kaaks, R.; Boeing, H.; Aleksandrova, K.; Trichopoulou, A.; Naska, A.; Orfanos, P.; Palli, D.; Agnoli, C.; Tumino, R.; Vineis, P.; Mattiello, A.; Braaten, T.; Borch, K.B.; Lund, E.; Menendez, V.; Sanchez, M.J.; Navarro, C.; Barricarte, A.; Amiano, P.; Sund, M.; Andersson, A.; Borgquist, S.; Olsson, A.; Khaw, K.T.; Wareham, N.; Travis, R.C.; Riboli, E.; Peeters, P.H.; May, A.M., Weight change in middle adulthood and breast cancer risk in the EPIC-PANACEA study. *Int. J. Cancer,* **2014**, *135*, (12), 2887-2899.

[20] Nagappan, T.; Ramasamy, P.; Wahid, M.E.; Segaran, T.C.; Vairappan, C.S., Biological activity of carbazole alkaloids and essential oil of Murraya koenigii against antibiotic resistant microbes and cancer cell lines. *Molecules,* **2011**, *16*, (11), 9651-9664.

[21] Sprague, B.L.; Trentham-Dietz, A.; Remington, P.L., The contribution of postmenopausal hormone use cessation to the declining incidence of breast cancer. *Cancer Causes Control,* **2011**, *22*, (1), 125-134.

[22] Normanno, N.; Morabito, A.; De Luca, A.; Piccirillo, M.C.; Gallo, M.; Maiello, M.R.; Perrone, F., Target-based therapies in breast

cancer: current status and future perspectives. *Endocr. Relat. Cancer,* **2009**, *16*, (3), 675-702.

[23] Mauri, D.; Polyzos, N.P.; Salanti, G.; Pavlidis, N.; Ioannidis, J.P., Multiple-treatments meta-analysis of chemotherapy and targeted therapies in advanced breast cancer. *J. Natl. Cancer Inst.,* **2008**, *100*, (24), 1780-1791.

[24] Fulda, S.; Meyer, E.; Friesen, C.; Susin, S.A.; Kroemer, G.; Debatin, K.M., Cell type specific involvement of death receptor and mitochondrial pathways in drug-induced apoptosis. *Oncogene,* **2001**, *20*, (9), 1063-1075.

[25] Ma, J.; Waxman, D.J., Combination of antiangiogenesis with chemotherapy for more effective cancer treatment. *Mol. Cancer Ther.,* **2008**, *7*, (12), 3670-3684.

[26] Iacopetta, D.; Lappano, R.; Cappello, A.R.; Madeo, M.; De Francesco, E.M.; Santoro, A.; Curcio, R.; Capobianco, L.; Pezzi, V.; Maggiolini, M.; Dolce, V., SLC37A1 gene expression is up-regulated by epidermal growth factor in breast cancer cells. *Breast Cancer Res. Treat.,* **2010**, *122*, (3), 755-764.

[27] Rechoum, Y.; Rovito, D.; Iacopetta, D.; Barone, I.; Ando, S.; Weigel, N.L.; O'Malley, B.W.; Brown, P.H.; Fuqua, S.A., AR collaborates with ERalpha in aromatase inhibitor-resistant breast cancer. *Breast Cancer Res. Treat.,* **2014**, *147*, (3), 473-485.

[28] Iacopetta, D.; Rechoum, Y.; Fuqua, S.A.W., The role of androgen receptor in breast cancer. *Drug Discovery Today: Disease Mechanisms,* **2012**, *Volume 9* (1-2), e19–e27.

[29] Miller, K.D., Recent translational research: antiangiogenic therapy for breast cancer - where do we stand? *Breast Cancer Res.,* **2004**, *6*, (3), 128-132.

[30] Liu, C.H.; Lin, C.; Tsai, K.J.; Chuang, Y.C.; Huang, Y.L.; Lee, T.H.; Huang, L.J.; Chan, H.C., Biological evaluation of 9-[(6-chloropyridin-4-yl)methyl]-9H-carbazole-3-carbinol as an anticancer agent. *Oncol. Rep.,* **2013**, *29*, (4), 1501-1509.

[31] Waldau, D.; Mikolasch, A.; Lalk, M.; Schauer, F., Derivatization of bioactive carbazoles by the biphenyl-degrading bacterium Ralstonia sp. strain SBUG 290. *Appl. Microbiol. Biotechnol.,* **2009**, *83*, (1), 67-75.

[32] Liu, Z.; Larock, R.C., Synthesis of Carbazoles and Dibenzofurans via Cross-Coupling of o-Iodoanilines and o-Iodophenols with Silylaryl Triflates and Subsequent Pd-Catalyzed Cyclization. *Tetrahedron,* **2007**, *63*, (2), 347-355.

[33] Stiborova, M.; Rupertova, M.; Schmeiser, H.H.; Frei, E., Molecular mechanisms of antineoplastic action of an anticancer drug ellipticine. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub,* **2006**, *150*, (1), 13-23.

[34] Wang, Y.M.; Hu, L.X.; Liu, Z.M.; You, X.F.; Zhang, S.H.; Qu, J.R.; Li, Z.R.; Li, Y.; Kong, W.J.; He, H.W.; Shao, R.G.; Zhang, L.R.; Peng, Z.G.; Boykin, D.W.; Jiang, J.D., N-(2,6-dimethoxypyridine-3-yl)-9-methylcarbazole-3-sulfonamide as a novel tubulin ligand against human cancer. *Clin. Cancer Res.,* **2008**, *14*, (19), 6218-6227.

[35] Tao, W.; South, V.J.; Zhang, Y.; Davide, J.P.; Farrell, L.; Kohl, N.E.; Sepp-Lorenzino, L.; Lobell, R.B., Induction of apoptosis by an inhibitor of the mitotic kinesin KSP requires both activation of the spindle assembly checkpoint and mitotic slippage. *Cancer Cell,* **2005**, *8*, (1), 49-59.

[36] Kops, G.J.; Weaver, B.A.; Cleveland, D.W., On the road to cancer: aneuploidy and the mitotic checkpoint. *Nat. Rev. Cancer,* **2005**, *5*, (10), 773-785.

[37] Scozzafava, A.; Owa, T.; Mastrolorenzo, A.; Supuran, C.T., Anticancer and antiviral sulfonamides. *Curr. Med. Chem.,* **2003**, *10*, (11), 925-953.

[38] Casini, A.; Scozzafava, A.; Mastrolorenzo, A.; Supuran, L.T., Sulfonamides and sulfonylated derivatives as anticancer agents. *Curr. Cancer Drug Targets,* **2002**, *2*, (1), 55-75.

[39] Panno, A.; Sinicropi, M.S.; Caruso, A.; El-Kashef, H.; Lancelot, J.C.; Aubert, G.; Lesnard, A.; Cresteil, T.; Rault, S., New Trimethoxybenzamides and Trimethoxyphenylureas Derived from Dimethylcarbazole as Cytotoxic Agents. Part I. *Journal of Heterocyclic Chemistry,* **2014**, *51*, (S1), E294–E302.

[40] Koyanagi, N.; Nagasu, T.; Fujita, F.; Watanabe, T.; Tsukahara, K.; Funahashi, Y.; Fujita, M.; Taguchi, T.; Yoshino, H.; Kitoh, K., *In vivo* tumor growth inhibition produced by a novel sulfonamide, E7010, against rodent and human tumors. *Cancer Res.,* **1994**, *54*, (7), 1702-1706.

[41] Hu, L.; Li, Z.R.; Li, Y.; Qu, J.; Ling, Y.H.; Jiang, J.D.; Boykin, D.W., Synthesis and structure-activity relationships of carbazole sulfonamides as a novel class of antimitotic agents against solid tumors. *J. Med. Chem.,* **2006**, *49*, (21), 6273-6282.

[42] Miller, K.D.; Sweeney, C.J.; Sledge, G.W., Jr., Redefining the target: chemotherapeutics as antiangiogenics. *J. Clin. Oncol.,* **2001**, *19*, (4), 1195-1206.

[43] Engler, T.A.; Furness, K.; Malhotra, S.; Sanchez-Martinez, C.; Shih, C.; Xie, W.; Zhu, G.; Zhou, X.; Conner, S.; Faul, M.M.; Sullivan, K.A.; Kolis, S.P.; Brooks, H.B.; Patel, B.; Schultz, R.M.; DeHahn, T.B.; Kirmani, K.; Spencer, C.D.; Watkins, S.A.; Considine, E.L.; Dempsey, J.A.; Ogg, C.A.; Stamm, N.B.; Anderson, B.D.; Campbell, R.M.; Vasudevan, V.; Lytle, M.L., Novel, potent and selective cyclin D1/CDK4 inhibitors: indolo[6,7-a]pyrrolo[3,4-c]carbazoles. *Bioorg. Med. Chem. Lett.,* **2003**, *13*, (14), 2261-2267.

[44] Yoon, S.; Kim, J.H.; Lee, Y.J.; Ahn, M.Y.; Choi, G.; Kim, W.K.; Yang, Z.; Lee, H.J.; Moon, H.R.; Kim, H.S., A novel carbazole derivative, MHY407, sensitizes cancer cells to doxorubicin-, etoposide-, and radiation treatment via DNA damage. *Eur. J. Pharmacol.,* **2012**, *697*, (1-3), 24-31.

[45] Kim, J.H.; Chae, M.; Kim, W.K.; Kim, Y.J.; Kang, H.S.; Kim, H.S.; Yoon, S., Salinomycin sensitizes cancer cells to the effects of doxorubicin and etoposide treatment by increasing DNA damage and reducing p21 protein. *Br. J. Pharmacol.,* **2011**, *162*, (3), 773-784.

[46] Kim, W.K.; Kim, J.H.; Yoon, K.; Kim, S.; Ro, J.; Kang, H.S.; Yoon, S., Salinomycin, a p-glycoprotein inhibitor, sensitizes radiation-treated cancer cells by increasing DNA damage and inducing G2 arrest. *Invest. New Drugs,* **2012**, *30*, (4), 1311-1318.

[47] Caruso, A.; Sinicropi, M.S.; Lancelot, J.C.; El-Kashef, H.; Saturnino, C.; Aubert, G.; Ballandonne, C.; Lesnard, A.; Cresteil, T.; Dallemagne, P.; Rault, S., Synthesis and evaluation of cytotoxic activities of new guanidines derived from carbazoles. *Bioorg. Med. Chem. Lett.,* **2014**, *24*, (2), 467-472.

[48] Kozurkova, M.; Sabolova, D.; Paulikova, H.; Janovec, L.; Kristian, P.; Bajdichova, M.; Busa, J.; Podhradsky, D.; Imrich, J., DNA binding properties and evaluation of cytotoxic activity of 9,10-bis-N-substituted (aminomethyl)anthracenes. *Int. J. Biol. Macromol.,* **2007**, *41*, (4), 415-422.

[49] Parisi, O.I.; Morelli, C.; Puoci, F.; Saturnino, C.; Caruso, A.; Sisci, D.; Trombino, G.E.; Picci, N.; Sinicropi, M.S., Magnetic molecularly imprinted polymers (MMIPs) for carbazole derivative release in targeted cancer therapy *Journal of Materials Chemistry B* **2014**, *2*, (38), 6619-6625.

[50] Svoboda, G.H.; Poore, G.A.; Montfort, M.L., Alkaloids of Ochrosia maculata Jacq. (Ochrosia borbonica Gmel.). Isolation of the alkaloids and study of the antitumor properties of 9-methoxyellipticine. *J. Pharm. Sci.,* **1968**, *57*, (10), 1720-1725.

[51] Hirata, K.; Ito, C.; Furukawa, H.; Itoigawa, M.; Cosentino, L.M.; Lee, K.H., Substituted 7H-pyrido[4,3-c]carbazoles with potent anti-HIV activity. *Bioorg. Med. Chem. Lett.,* **1999**, *9*, (2), 119-122.

[52] Mastalarz, H.; Jasztold-Howorko, R.; Rulko, F.; Croisy, A.; Carrez, D., Synthesis and cytostatic properties of some 6H-Indolo[2, 3-b][1, 8]naphthyridine derivatives. *Arch. Pharm. (Weinheim),* **2004**, *337*, (8), 434-439.

[53] Harding, M.M.; Grummitt, A.R., 9-hydroxyellipticine and derivatives as chemotherapy agents. *Mini Rev. Med. Chem.,* **2003**, *3*, (2), 67-76.

[54] Romero, M.; Renard, P.; Caignard, D.H.; Atassi, G.; Solans, X.; Constans, P.; Bailly, C.; Pujol, M.D., Synthesis and structure-activity relationships of new benzodioxinic lactones as potential anticancer drugs. *J. Med. Chem.,* **2007**, *50*, (2), 294-307.

[55] Prudent, R.; Moucadel, V.; Nguyen, C.H.; Barette, C.; Schmidt, F.; Florent, J.C.; Lafanechere, L.; Sautel, C.F.; Duchemin-Pelletier, E.; Spreux, E.; Filhol, O.; Reiser, J.B.; Cochet, C., Antitumor activity of pyridocarbazole and benzopyridoindole derivatives that inhibit protein kinase CK2. *Cancer Res.,* **2010**, *70*, (23), 9865-9874.

[56] Rouesse, J.; Spielmann, M.; Turpin, F.; Le Chevalier, T.; Azab, M.; Mondesir, J.M., Phase II study of elliptinium acetate salvage treatment of advanced breast cancer. *Eur. J. Cancer,* **1993**, *29A*, (6), 856-859.

[57] Le Pecq, J.B.; Nguyen Dat, X.; Gosse, C.; Paoletti, C., A new antitumoral agent: 9-hydroxyellipticine. Possibility of a rational

design of anticancerous drugs in the series of DNA intercalating drugs. *Proc. Natl. Acad. Sci. U. S. A.,* **1974**, *71*, (12), 5078-5082.

[58] Saravanabhavan, M.; Murugesan, V.; Sekar, M., Microwave assisted synthesis of pyrido[2,3-a]carbazoles; investigation of *in vitro* DNA binding/cleavage, antioxidant and cytotoxicity studies. *J. Photochem. Photobiol. B,* **2014**, *133*, 145-152.

[59] Knolker, H.J.; Reddy, K.R., Isolation and synthesis of biologically active carbazole alkaloids. *Chem. Rev.,* **2002**, *102*, (11), 4303-4427.

[60] Tsuchimoto, T.; Matsubayashi, H.; Kaneko, M.; Shirakawa, E.; Kawakami, Y., Easy access to aryl- and heteroaryl-annulated[a]carbazoles by the indium-catalyzed reaction of 2-arylindoles with propargyl ethers. *Angew. Chem. Int. Ed. Engl.,* **2005**, *44*, (9), 1336-1340.

[61] Martinez-Esperon, M.F.; Rodriguez, D.; Castedo, L.; Saa, C., Synthesis of carbazoles from ynamides by intramolecular dehydro Diels-Alder reactions. *Org. Lett.,* **2005**, *7*, (11), 2213-2216.

[62] Rouesse, J.G.; Le Chevalier, T.; Caille, P.; Mondesir, J.M.; Sancho-Garnier, H.; May-Levin, F.; Spielmann, M.; De Jager, R.; Amiel, J.L., Phase II study of elliptinium in advanced breast cancer. *Cancer Treat. Rep.,* **1985**, *69*, (6), 707-708.

[63] Moody, T.W.; Czerwinski, G.; Tarasova, N.I.; Michejda, C.J., VIP-ellipticine derivatives inhibit the growth of breast cancer cells. *Life Sci.,* **2002**, *71*, (9), 1005-1014.

[64] Kuo, P.L.; Hsu, Y.L.; Kuo, Y.C.; Chang, C.H.; Lin, C.C., The anti-proliferative inhibition of ellipticine in human breast mda-mb-231 cancer cells is through cell cycle arrest and apoptosis induction. *Anticancer. Drugs,* **2005**, *16*, (7), 789-795.

[65] Arguello, F.; Alexander, M.A.; Greene, J.F., Jr.; Stinson, S.F.; Jorden, J.L.; Smith, E.M.; Kalavar, N.T.; Alvord, W.G.; Klabansky, R.L.; Sausville, E.A., Preclinical evaluation of 9-chloro-2-methylellipticinium acetate alone and in combination with conventional anticancer drugs for the treatment of human brain tumor xenografts. *J. Cancer Res. Clin. Oncol.,* **1998**, *124*, (1), 19-26.

[66] Goodwin, S.; Smith, A.F.; Horning, E.C., Alkaloids of Ochrosia elliptica Labill. J. *J. Am. Chem. Soc.* . **1959**, *81*, 1903–1908.

[67] Treat, J.; Greenspan, A.; Rahman, A.; McCabe, M.S.; Byrne, P.J., Elliptinium: phase II study in advanced measurable breast cancer. *Invest. New Drugs,* **1989**, *7*, (2-3), 231-234.

[68] Buzdar, A.U.; Hortobagyi, G.N.; Esparza, L.T.; Holmes, F.A.; Ro, J.S.; Fraschini, G.; Lichtiger, B., Elliptinium acetate in metastatic breast cancer--a phase II study. *Oncology,* **1990**, *47*, (2), 101-104.

[69] Dodin, G.; Andrieux, M.; al Kabbani, H., Binding of ellipticine to beta-lactoglobulin. A physico-chemical study of the specific interaction of an antitumor drug with a transport protein. *Eur. J. Biochem.,* **1990**, *193*, (3), 697-700.

[70] Kovacic, P.; Ames, J.R.; Lumme, P.; Elo, H.; Cox, O.; Jackson, H.; Rivera, L.A.; Ramirez, L.; Ryan, M.D., Charge transfer-oxy radical mechanism for anti-cancer agents. *Anticancer. Drug Des.,* **1986**, *1*, (3), 197-214.

[71] Lesca, P.; Rafidinarivo, E.; Lecointe, P.; Mansuy, D., A class of strong inhibitors of microsomal monooxygenases: the ellipticines. *Chem. Biol. Interact.,* **1979**, *24*, (2), 189-197.

[72] Schwaller, M.A.; Allard, B.; Lescot, E.; Moreau, F., Protonophoric activity of ellipticine and isomers across the energy-transducing membrane of mitochondria. *J. Biol. Chem.,* **1995**, *270*, (39), 22709-22713.

[73] Peng, Y.; Li, C.; Chen, L.; Sebti, S.; Chen, J., Rescue of mutant p53 transcription function by ellipticine. *Oncogene,* **2003**, *22*, (29), 4478-4487.

[74] Kuo, P.L.; Hsu, Y.L.; Chang, C.H.; Lin, C.C., The mechanism of ellipticine-induced apoptosis and cell cycle arrest in human breast MCF-7 cancer cells. *Cancer Lett.,* **2005**, *223*, (2), 293-301.

[75] Monnot, M.; Mauffret, O.; Simon, V.; Lescot, E.; Psaume, B.; Saucier, J.M.; Charra, M.; Belehradek, J., Jr.; Fermandjian, S., DNA-drug recognition and effects on topoisomerase II-mediated cytotoxicity. A three-mode binding model for ellipticine derivatives. *J. Biol. Chem.,* **1991**, *266*, (3), 1820-1829.

[76] Singh, M.P.; Hill, G.C.; Peoc'h, D.; Rayner, B.; Imbach, J.L.; Lown, J.W., High-field NMR and restrained molecular modeling studies on a DNA heteroduplex containing a modified apurinic abasic site in the form of covalently linked 9-aminoellipticine. *Biochemistry (Mosc.),* **1994**, *33*, (34), 10271-10285.

[77] Fosse, P.; Rene, B.; Charra, M.; Paoletti, C.; Saucier, J.M., Stimulation of topoisomerase II-mediated DNA cleavage by ellipticine derivatives: structure-activity relationship. *Mol. Pharmacol.,* **1992**, *42*, (4), 590-595.

[78] Froelich-Ammon, S.J.; Patchan, M.W.; Osheroff, N.; Thompson, R.B., Topoisomerase II binds to ellipticine in the absence or presence of DNA. Characterization of enzyme-drug interactions by fluorescence spectroscopy. *J. Biol. Chem.,* **1995**, *270*, (25), 14998-15004.

[79] Tmejova, K.; Krejcova, L.; Hynek, D.; Adam, V.; Babula, P.; Trnkova, L.; Stiborova, M.; Eckschlager, T.; Kizek, R., Electrochemical study of ellipticine interaction with single and double stranded oligonucleotides. *Anticancer Agents Med. Chem.,* **2014**, *14*, (2), 331-340.

[80] Sengupta, A.; Koninti, R.K.; Gavvala, K.; Ballav, N.; Hazra, P., An anticancer drug to probe non-specific protein-DNA interactions. *Phys. Chem. Chem. Phys.,* **2014**, *16*, (9), 3914-3917.

[81] Ryvolova, M.; Adam, V.; Eckschlager, T.; Stiborova, M.; Kizek, R., Study of DNA-ellipticine interaction by capillary electrophoresis with laser-induced fluorescence detection. *Electrophoresis,* **2012**, *33*, (11), 1545-1549.

[82] Stiborova, M.; Borek-Dohalska, L.; Aimova, D.; Kotrbova, V.; Kukackova, K.; Janouchova, K.; Rupertova, M.; Ryslava, H.; Hudecek, J.; Frei, E., Oxidation pattern of the anticancer drug ellipticine by hepatic microsomes - similarity between human and rat systems. *Gen. Physiol. Biophys.,* **2006**, *25*, (3), 245-261.

[83] Stiborova, M.; Sejbal, J.; Borek-Dohalska, L.; Aimova, D.; Poljakova, J.; Forsterova, K.; Rupertova, M.; Wiesner, J.; Hudecek, J.; Wiessler, M.; Frei, E., The anticancer drug ellipticine forms covalent DNA adducts, mediated by human cytochromes P450, through metabolism to 13-hydroxyellipticine and ellipticine N2-oxide. *Cancer Res.,* **2004**, *64*, (22), 8374-8380.

[84] Stiborova, M.; Frei, E., Ellipticines as DNA-targeted chemotherapeutics. *Curr. Med. Chem.,* **2014**, *21*, (5), 575-591.

[85] Chang, C.Y.; Puga, A., Constitutive activation of the aromatic hydrocarbon receptor. *Mol. Cell. Biol.,* **1998**, *18*, (1), 525-535.

[86] Ivy, S.P.; Tulpule, A.; Fairchild, C.R.; Averbuch, S.D.; Myers, C.E.; Nebert, D.W.; Baird, W.M.; Cowan, K.H., Altered regulation of P-450IA1 expression in a multidrug-resistant MCF-7 human breast cancer cell line. *J. Biol. Chem.,* **1988**, *263*, (35), 19119-19125.

[87] Deane, F.M.; O'Sullivan, E.C.; Maguire, A.R.; Gilbert, J.; Sakoff, J.A.; McCluskey, A.; McCarthy, F.O., Synthesis and evaluation of novel ellipticines as potential anti-cancer agents. *Org. Biomol. Chem.,* **2013**, *11*, (8), 1334-1344.

[88] Miller, C.M.; O'Sullivan, E.C.; Devine, K.J.; McCarthy, F.O., Synthesis and biological evaluation of novel isoellipticine derivatives and salts. *Org. Biomol. Chem.,* **2012**, *10*, (39), 7912-7921.

[89] Ghosh, S.; Kar, A.; Chowdhury, S.; Dasgupta, D., Ellipticine binds to a human telomere sequence: an additional mode of action as a putative anticancer agent? *Biochemistry (Mosc.),* **2013**, *52*, (24), 4127-4137.

[90] Renault, G.; Malvy, C.; Venegas, W.; Larsen, A.K., *In vivo* exposure to four ellipticine derivatives with topoisomerase inhibitory activity results in chromosome clumping and sister chromatid exchange in murine bone marrow cells. *Toxicol. Appl. Pharmacol.,* **1987**, *89*, (2), 281-286.

[91] Sato, N.; Mizumoto, K.; Kusumoto, M.; Niiyama, H.; Maehara, N.; Ogawa, T.; Tanaka, M., 9-Hydroxyellipticine inhibits telomerase activity in human pancreatic cancer cells. *FEBS Lett.,* **1998**, *441*, (2), 318-321.

[92] Lesca, P.; Lecointe, P.; Paoletti, C.; Mansuy, D., Ellipticines as potent inhibitors of aryl hydrocarbon hydroxylase: their binding to microsomal cytochromes P450 and protective effect against benzo(a)pyrene mutagenicity. *Biochem. Pharmacol.,* **1978**, *27*, (8), 1203-1209.

[93] Ohashi, M.; Sugikawa, E.; Nakanishi, N., Inhibition of p53 protein phosphorylation by 9-hydroxyellipticine: a possible anticancer mechanism. *Jpn. J. Cancer Res.,* **1995**, *86*, (9), 819-827.

[94] Sugikawa, E.; Hosoi, T.; Yazaki, N.; Gamanuma, M.; Nakanishi, N.; Ohashi, M., Mutant p53 mediated induction of cell cycle arrest and apoptosis at G1 phase by 9-hydroxyellipticine. *Anticancer Res.,* **1999**, *19*, (4B), 3099-3108.

[95] Andrews, W.J.; Panova, T.; Normand, C.; Gadal, O.; Tikhonova, I.G.; Panov, K.I., Old drug, new target: ellipticines selectively

inhibit RNA polymerase I transcription. *J. Biol. Chem.,* **2013**, *288*, (7), 4567-4582.

[96] Pandrangi, S.L.; Chikati, R.; Chauhan, P.S.; Kumar, C.S.; Banarji, A.; Saxena, S., Effects of ellipticine on ALDH1A1-expressing breast cancer stem cells--an *in vitro* and in silico study. *Tumour Biol.,* **2014**, *35*, (1), 723-737.

[97] Micalizzi, D.S.; Farabaugh, S.M.; Ford, H.L., Epithelial-mesenchymal transition in cancer: parallels between normal development and tumor progression. *J. Mammary Gland Biol. Neoplasia,* **2010**, *15*, (2), 117-134.

[98] Li, T.; Li, Y.G.; Pu, D.M., Matrix metalloproteinase-2 and -9 expression correlated with angiogenesis in human adenomyosis. *Gynecol. Obstet. Invest.,* **2006**, *62*, (4), 229-235.

[99] Hung, W.C.; Chang, H.C., Indole-3-carbinol inhibits Sp1-induced matrix metalloproteinase-2 expression to attenuate migration and invasion of breast cancer cells. *J. Agric. Food Chem.,* **2009**, *57*, (1), 76-82.

[100] Radisky, E.S.; Radisky, D.C., Matrix metalloproteinase-induced epithelial-mesenchymal transition in breast cancer. *J. Mammary Gland Biol. Neoplasia,* **2010**, *15*, (2), 201-212.

[101] Ho, J.N.; Jun, W.; Choue, R.; Lee, J., I3C and ICZ inhibit migration by suppressing the EMT process and FAK expression in breast cancer cells. *Mol Med Rep,* **2013**, *7*, (2), 384-388.

[102] Luo, M.; Guan, J.L., Focal adhesion kinase: a prominent determinant in breast cancer initiation, progression and metastasis. *Cancer Lett.,* **2010**, *289*, (2), 127-139.

[103] Zhao, X.; Guan, J.L., Focal adhesion kinase and its signaling pathways in cell migration and angiogenesis. *Adv Drug Deliv Rev,* **2011**, *63*, (8), 610-615.

[104] Chen, A.Y.; Shih, S.J.; Hsiao, M.; Rothenberg, M.L.; Prudhomme, M., Induction of radiosensitization by indolocarbazole derivatives: the role of DNA topoisomerase I. *Mol. Pharmacol.,* **2004**, *66*, (3), 553-560.

[105] Yamashita, Y.; Fujii, N.; Murakata, C.; Ashizawa, T.; Okabe, M.; Nakano, H., Induction of mammalian DNA topoisomerase I mediated DNA cleavage by antitumor indolocarbazole derivatives. *Biochemistry (Mosc.),* **1992**, *31*, (48), 12069-12075.

[106] Yoshinari, T.; Yamada, A.; Uemura, D.; Nomura, K.; Arakawa, H.; Kojiri, K.; Yoshida, E.; Suda, H.; Okura, A., Induction of topoisomerase I-mediated DNA cleavage by a new indolocarbazole, ED-110. *Cancer Res.,* **1993**, *53*, (3), 490-494.

[107] Labourier, E.; Riou, J.F.; Prudhomme, M.; Carrasco, C.; Bailly, C.; Tazi, J., Poisoning of topoisomerase I by an antitumor indolocarbazole drug: stabilization of topoisomerase I-DNA covalent complexes and specific inhibition of the protein kinase activity. *Cancer Res.,* **1999**, *59*, (1), 52-55.

[108] Chen, A.Y.; Choy, H.; Rothenberg, M.L., DNA topoisomerase I-targeting drugs as radiation sensitizers. *Oncology (Williston Park),* **1999**, *13*, (10 Suppl 5), 39-46.

[109] Burstein, H.J.; Overmoyer, B.; Gelman, R.; Silverman, P.; Savoie, J.; Clarke, K.; Dumadag, L.; Younger, J.; Ivy, P.; Winer, E.P., Rebeccamycin analog for refractory breast cancer: a randomized phase II trial of dosing schedules. *Invest. New Drugs,* **2007**, *25*, (2), 161-164.

[110] Marminon, C.; Anizon, F.; Moreau, P.; Pfeiffer, B.; Pierre, A.; Golsteyn, R.M.; Peixoto, P.; Hildebrand, M.P.; David-Cordonnier, M.H.; Lozach, O.; Meijer, L.; Prudhomme, M., Rebeccamycin derivatives as dual DNA-damaging agents and potent checkpoint kinase 1 inhibitors. *Mol. Pharmacol.,* **2008**, *74*, (6), 1620-1629.

[111] Marminon, C.; Pierre, A.; Pfeiffer, B.; Perez, V.; Leonce, S.; Joubert, A.; Bailly, C.; Renard, P.; Hickman, J.; Prudhomme, M., Syntheses and antiproliferative activities of 7-azarebeccamycin analogues bearing one 7-azaindole moiety. *J. Med. Chem.,* **2003**, *46*, (4), 609-622.

[112] Fracasso, P.M.; Williams, K.J.; Chen, R.C.; Picus, J.; Ma, C.X.; Ellis, M.J.; Tan, B.R.; Pluard, T.J.; Adkins, D.R.; Naughton, M.J.; Rader, J.S.; Arquette, M.A.; Fleshman, J.W.; Creekmore, A.N.; Goodner, S.A.; Wright, L.P.; Guo, Z.; Ryan, C.E.; Tao, Y.; Soares, E.M.; Cai, S.R.; Lin, L.; Dancey, J.; Rudek, M.A.; McLeod, H.L.; Piwnica-Worms, H., A Phase 1 study of UCN-01 in combination with irinotecan in patients with resistant solid tumor malignancies. *Cancer Chemother. Pharmacol.,* **2011**, *67*, (6), 1225-1237.

[113] Ma, C.X.; Ellis, M.J.; Petroni, G.R.; Guo, Z.; Cai, S.R.; Ryan, C.E.; Craig Lockhart, A.; Naughton, M.J.; Pluard, T.J.; Brenin, C.M.; Picus, J.; Creekmore, A.N.; Mwandoro, T.; Yarde, E.R.; Reed, J.; Ebbert, M.; Bernard, P.S.; Watson, M.; Doyle, L.A.; Dancey, J.; Piwnica-Worms, H.; Fracasso, P.M., A phase II study of UCN-01 in combination with irinotecan in patients with metastatic triple negative breast cancer. *Breast Cancer Res. Treat.,* **2013**, *137*, (2), 483-492.

[114] Sun, Y.; Li, Y.X.; Wu, H.J.; Wu, S.H.; Wang, Y.A.; Luo, D.Z.; Liao, D.J., Effects of an Indolocarbazole-Derived CDK4 Inhibitor on Breast Cancer Cells. *J Cancer,* **2011**, *2*, 36-51.

[115] Ohkubo, M.; Nishimura, T.; Honma, T.; Nishimura, I.; Ito, S.; Yoshinari, T.; Suda, H.A.; Morishima, H.; Nishimura, S., Synthesis and biological activities of NB-506 analogues: Effects of the positions of two hydroxyl groups at the indole rings. *Bioorg. Med. Chem. Lett.,* **1999**, *9*, (23), 3307-3312.

[116] Ciomei, M.; Croci, V.; Stellari, F.; Amboldi, N.; Giavarini, R.; Pesenti, E., Antitumor activity of edotecarin in breast carcinoma models. *Cancer Chemother. Pharmacol.,* **2007**, *60*, (2), 229-235.

[117] Faneyte, I.F.; Kristel, P.M.; Maliepaard, M.; Scheffer, G.L.; Scheper, R.J.; Schellens, J.H.; van de Vijver, M.J., Expression of the breast cancer resistance protein in breast cancer. *Clin. Cancer Res.,* **2002**, *8*, (4), 1068-1074.

[118] Cavailles, V.; Gompel, A.; Portois, M.C.; Thenot, S.; Mabon, N.; Vignon, F., Comparative activity of pulsed or continuous estradiol exposure on gene expression and proliferation of normal and tumoral human breast cells. *J. Mol. Endocrinol.,* **2002**, *28*, (3), 165-175.

[119] Kubota, T.; Josui, K.; Fukutomi, T.; Kitajima, M., Growth regulation by estradiol, progesterone and recombinant human epidermal growth factor of human breast carcinoma xenografts grown serially in nude mice. *Anticancer Res.,* **1995**, *15*, (4), 1275-1278.

[120] Arakawa, H.; Morita, M.; Kodera, T.; Okura, A.; Ohkubo, M.; Morishima, H.; Nishimura, S., *In vivo* anti-tumor activity of a novel indolocarbazole compound, J-107088, on murine and human tumors transplanted into mice. *Jpn. J. Cancer Res.,* **1999**, *90*, (10), 1163-1170.

[121] Nakamura, K.; Uenaka, T.; Nagasu, T.; Sugumi, H.; Yamaguchi, A.; Kotake, Y.; Okada, T.; Kamata, J.; Niijima, J.; Taniguchi, T.; Koyanagi, N.; Yoshino, H.; Kitoh, K.; Yoshimatsu, K., A novel carbazole topoisomerase II poison, ER-37328: potent tumoricidal activity against human solid tumors *in vitro* and *in vivo*. *Cancer Sci.,* **2003**, *94*, (1), 119-124.

[122] Kouadio, K.; Chenieux, J.C.; Rideau, M.; Viel, C., Antitumor alkaloids in callus cultures of Ochrosia elliptica. *J. Nat. Prod.,* **1984**, *47*, (5), 872-874.

Received: ????????, 2015 Revised: ????????, 2015 Accepted: ??????????, 2015