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REVIEW ARTICLE



## Carbazole scaffolds in cancer therapy: a review from 2012 to 2018

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### ABSTRACT

For over half a century, the carbazole skeleton has been the key structural motif of many biologically active compounds including natural and synthetic products. Carbazoles have taken an important part in all the existing anti-cancer drugs because of their discovery from a large variety of organisms, including bacteria, fungi, plants, and animals. In this article, we specifically explored the literature from 2012 to 2018 on the anti-tumour activities reported to carbazole derivatives and we have critically collected the most significant data. The most described carbazole anti-tumour agents were classified according to their structure, starting from the tricyclic-carbazole motif to fused tetra-, penta-, hexa- and heptacyclic carbazoles. To date, three derivatives are available on the market and approved in cancer therapy.

### ARTICLE HISTORY

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### KEYWORDS





Carbazole; cancer; cytotoxicity; targeted therapy; enzyme inhibitors

## 1. Introduction

Cancer is characterized by an uncontrolled growth of cells, which can spread to distant sites of the body with severe health consequences and is the second leading cause of death worldwide<sup>1</sup>. Around 14.1 million new cancer cases and 8.2 million cancer-related deaths occurred in 2012, and 29.4 million new cases are estimated for 2035 with 18.8 million cancer-related deaths (GLOBOCAN 2012)<sup>2</sup>. 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. Keeping in mind that both cancer cases are increasing and resistance to anti-cancer drug regimens are emerging, research and development of new powerful cancer treatments became extremely crucial for the next decades. Among the existing anti-cancer drugs, the carbazole scaffolds have been, for over half a century, the key structural motif of many biologically active compounds including natural and synthetic products<sup>3</sup>. Carbazole alkaloids originate in most cases from higher plants of the genera *Murraya*, *Glycosmis*, *Clausena* and *Micromelum*, all from the family of Rutaceae<sup>4</sup>. Other sources are bacteria (e.g. *Streptomyces*), algae (e.g. *Hyella caespitosa*) and fungi (e.g. *Aspergillus* species). The parent compound 9H-carbazole was isolated from coal tar in 1872 by Graebe and Glazer<sup>5</sup>. The first naturally occurring carbazole, the alkaloid murrayanine, was isolated from *Murraya koenigii* Spreng in 1962<sup>6</sup>. Later, many carbazole derivatives have been synthesized and are well known for their pharmacological activities such as anti-oxidant, anti-inflammatory, anti-bacterial, anti-tumour, anti-convulsant, anti-psychotic and

anti-diabetic<sup>7</sup>. Many carbazole derivatives and related compounds have been studied. More interestingly, three derivatives have obtained marketing authorization with anti-cancer drug status in different countries. Ellipticine, which was discovered in 1959 (Figure 1) and extracted from the leaves of *Ochrosia elliptica* (Apocynaceae) before being entirely synthesized, could be considered as the first initial lead compound of carbazole analogues. Thereafter, an ellipticine analogue named *N*-methyl-9-hydroxyellipticinium acetate (Celiptium<sup>®</sup>) has been developed. Since 1982, Celiptium<sup>®</sup> is still currently used in the treatment of metastatic breast cancer<sup>8,9</sup>. As reported by the National Cancer Institute (NCI) drug dictionary<sup>10</sup>, *N*-methyl-9-hydroxyellipticinium acetate acted as a topoisomerase II inhibitor and an intercalating agent, stabilizing the cleavable complex of topoisomerase II and inducing DNA breakages, thereby inhibiting DNA replication and RNA and protein synthesis. New *N*-thioalkylcarbazole derivatives were synthesized and evaluated in comparison to ellipticine. Among the bioactive carbazole-type derivatives, 7-(6-bromo-1,4-dimethyl-9H-carbazol-9-yl)-heptane-1-thiol (Figure 1) needs to be also mentioned<sup>9</sup>.

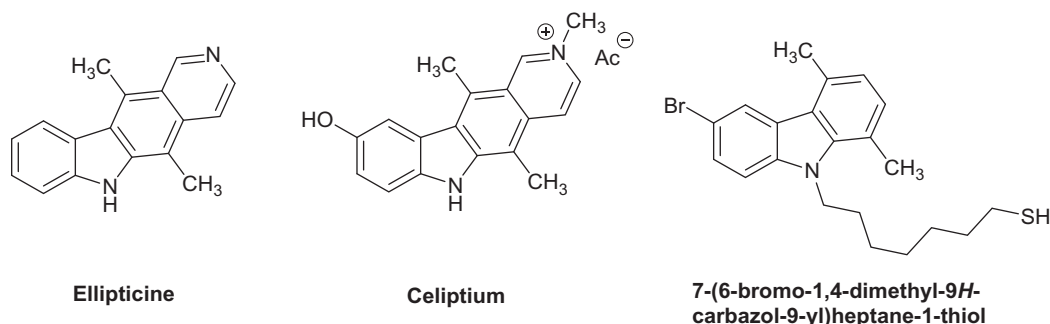
The second derivative to obtain marketing authorization was alectinib bearing a 5H-benzo[b]carbazol-11(6H)-one scaffold (AF802, CH 5424802, RG7853, RO5424802, Alecensa<sup>®</sup>) (Figure 2). Alectinib, an orally available drug, was first approved in 2015 by the US Food and Drug Administration (FDA)<sup>11</sup> for Genentech and then by the European Medicines Agency (EMA) for Roche Pharmaceuticals<sup>12</sup>, with an indication as monotherapy for the treatment of adult patients with anaplastic lymphoma kinase (ALK)-positive advanced non-small-cell lung cancer (NSCLC)<sup>13</sup>.

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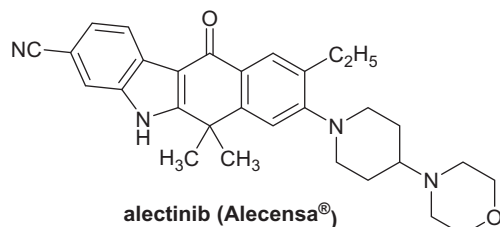
\*These authors contributed equally to this article.

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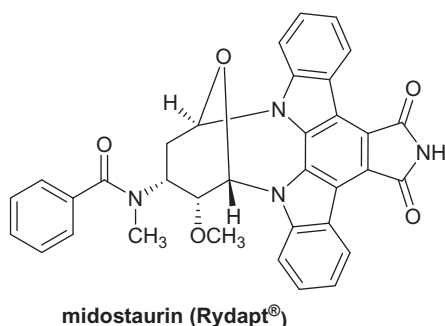
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**Figure 1.** Chemical structures of ellipticine, elliptinium acetate (Celiptium®) and a 6-bromo derivative of carbazole.



**Figure 2.** Chemical structure of alectinib, an ALK inhibitor.



**Figure 3.** Chemical structure of midostaurin, an FLT3 inhibitor.

The third derivative recently approved in 2017 by the FDA<sup>14</sup> and the EMA<sup>15</sup> is midostaurin (CGP41251, PKC412, Rydapt®) (Novartis) (Figure 3), described mainly as the first fms-like tyrosine kinase 3 (FLT3) inhibitor for newly diagnosed acute myeloid leukemia (AML) and for advanced systemic mastocytosis (SM)<sup>16,17</sup>.

Compared to the previously recent published reviews<sup>18</sup>, we focused this article on the carbazole derivatives exerting anti-tumour activity reported from 2012 to 2018, and we critically collected the most significant data. The term “carbazole” includes both the tricyclic molecular skeleton and diverse fused carbazoles including tetracyclic (with 5-, 6- and 7-membered rings), pentacyclic, hexacyclic and finally heptacyclic fused carbazoles (Figure 4). Several databases, bibliographic information (articles) from namely ScienceDirect, Scifinder, Pubmed and Web of Science as well as technological (patents) information from INPI Patents Database, European Patent Office (EPO), as well as the World Intellectual Property Organization (WIPO), were used as literature sources.

The increased interest in the use of carbazole derivatives for the cancer therapy can also be expressed in the research of patents. In Table 1, the publication of 10 patents<sup>19–28</sup> is summarized including one European patent, three US patents and seven international patents for carbazole derivatives currently described for their anti-cancer activity.

## 2. Tricyclic carbazoles

### 2.1. Ferrocenyl platinum(II) complex

Ferrocenyl platinum(II) complex [Pt(Fc-tpy)(NPC)]Cl (HNPC = N-propargylcarbazole) was synthesized and evaluated for its anti-proliferative properties in visible light against HaCaT (human keratinocyte) cell lines. Compound **1** (Figure 5) exhibited interesting photocytotoxicity in HaCaT cell lines with an IC<sub>50</sub> value of 12.0 μM in visible light (400–700 nm) with low dark toxicity (IC<sub>50</sub> > 60 μM)<sup>29</sup>.

### 2.2. N-Acylcarbazoles

N-Acylated carbazoles were synthesized and evaluated for their anti-proliferative activities against CAL 27 (squamous cell carcinoma) cell lines. The IC<sub>50</sub> values of the most active compounds **2a** and **2b** (Figure 6) were 0.028 and 0.45 μM, respectively<sup>30</sup>.

### 2.3. 6-Methyl-2,3,4,9-tetrahydro-1H-carbazoles

Tetrahydrocarbazoles were synthesized and tested for anti-cancer activity against six different cell lines, namely human kidney adenocarcinoma (ACHN), pancreas carcinoma (Panc1), lung carcinoma (GIII and Calu1), non-small-cell lung carcinoma (H460), human colon carcinoma (HCT116) and normal breast epithelium (MCF10A) cell lines. Carbazole derivatives demonstrated moderate to good activities and among them, compound **3** (Figure 7) was found to be the most active against Calu1 cell line with an IC<sub>50</sub> of 2.5 nM<sup>31</sup>.

### 2.4. N-{3-[3-(9-Methyl-9H-carbazol-3-yl)-acryloyl]-phenyl}-benzamide

These derivatives (Figure 8) were synthesized and evaluated for their in vitro xanthine oxidase (XO), tyrosinase and melanin production inhibitory activity. Most of the target compounds (**4a**, **4c**, **4d**, **4e**, **4g**, **4i** and **4j**) inhibited XO with IC<sub>50</sub> values comprised between 4.3 and 5.6 μM. Furthermore, these derivatives showed a better activity than the standard drug allopurinol (IC<sub>50</sub> value of 8.5 μM). Interestingly, compound **4a** bearing a cyclopropyl ring was found to be the most potent inhibitor of XO with an IC<sub>50</sub> of 4.3 μM. Compounds **4b**, **4d**, **4f**, **4h** and **4j** were found to be potent inhibitors of tyrosinase (IC<sub>50</sub> values ranging from 14.01 to 17.52 μM). These results suggest the possible use of these compounds for the design and development of novel XO and tyrosinase inhibitors<sup>32</sup>.

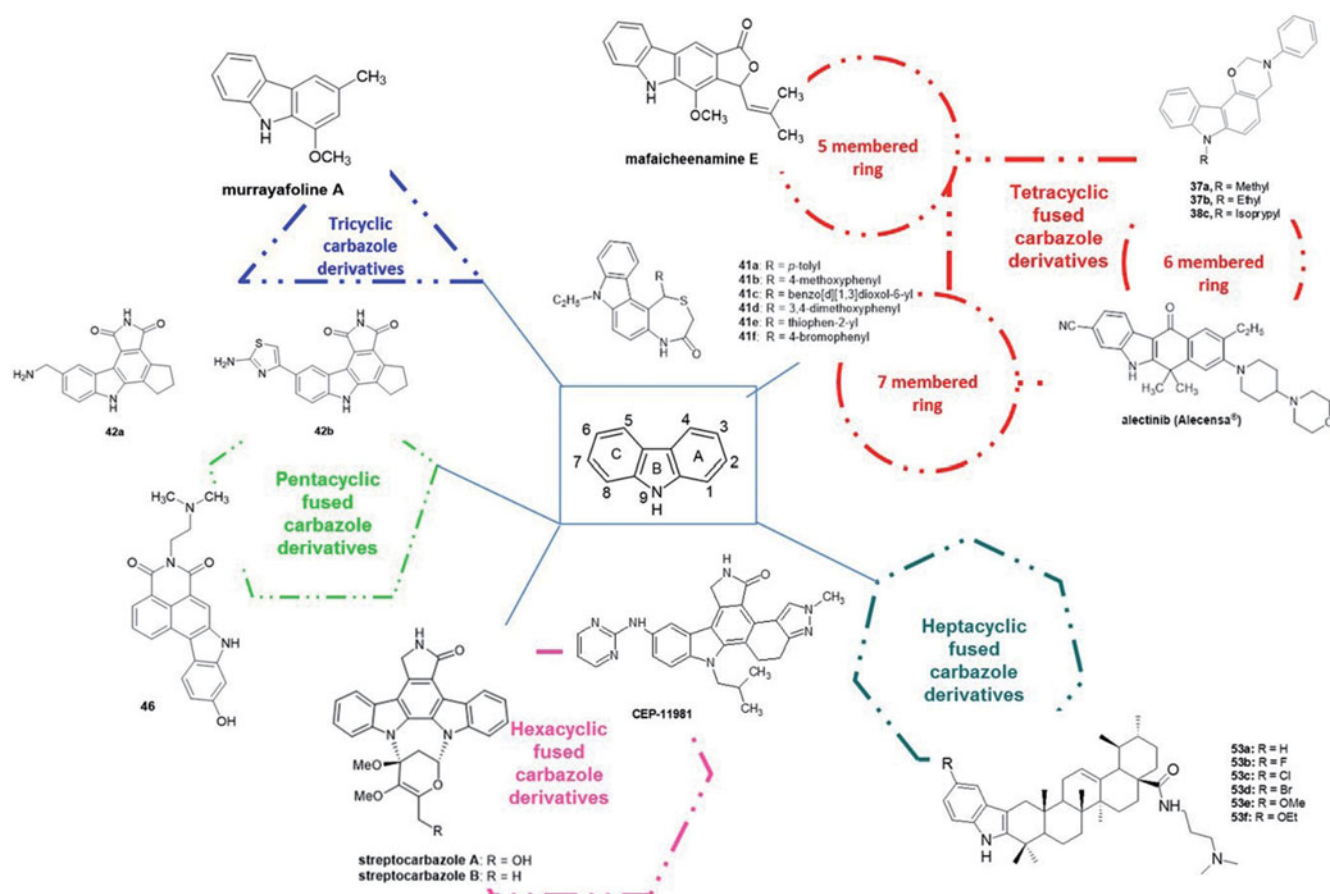


Figure 4. Main frameworks of biologically active carbazole alkaloids.

### 2.5. 3,6-Bis(1-methyl-4-vinylpyridinium) carbazole diiodide (BMVC)

BMVC or compound **5** (Figure 9) is known for its ability (i) to suppress the telomerase activity, (ii) to induce senescence of cancer cells and (iii) to destroy the intra-tumour vasculature. BMVC was studied for tumour targeting as well as for its photo-induced anti-tumour effect. The properties of this fluorescent molecule provided a design of photosensitizer (PS) for photodynamic therapy (PDT) treatment. PDT results showed that BMVC inhibited the growth of tumour cells both in vitro and in vivo<sup>33</sup>. BMVC is the most studied to this date as “G-quadruplex” ligand, which interacted with different forms of nucleic acids and stabilizes G-quadruplex structures. BMVC suppressed the tumour-related properties of cancer cells, including cell migration, colony-forming ability and anchorage-independent growth<sup>34</sup>. In clinical tests (overall, 114 outpatients), the use of fluorescent BMVC was investigated for the cancer diagnosis (needle aspirates of neck masses)<sup>35</sup>. Many analogue derivatives of BMVC have been analysed and used as probes due to the fluorescent electron donating optical chromophore properties of this carbazole derivative. Recently a new derivative has been used for the detection of bcl-2 2345 quadruplex structures<sup>36</sup>.

### 2.6. Benzopsoralen and 3-hydroxy-N-alkylcarbazole

Benzopsoralen derivatives and its carbazole analogues were synthesized, tested against MDA MB231 (breast carcinoma) and TCC-SUP (urinary bladder cell carcinoma) cell lines, and their

mechanism of action was investigated by means of molecular docking studies. Every benzopsoralen and carbazole derivative showed interesting anti-proliferative activities, with  $GI_{50}$  values in the nanomolar range against both cell lines. Among carbazole derivatives, compound **6** (Figure 10) had very strong activity with  $GI_{50}$  values of 0.198 and 0.025  $\mu$ M against MDA MB231 and TCC-SUP cell lines, respectively<sup>37</sup>.

### 2.7. MHY407

The carbazole derivative **MHY407** (Figure 11) is active against breast cancer cell lines by inhibiting cellular proliferation with  $IC_{50}$  around 5  $\mu$ M. This compound increased DNA damage and triggered cell cycle arrest in S phase. In combination with various chemotherapeutic treatments, such as doxorubicin, etoposide or radiation, **MHY407** improved the efficiency of the treatments by reducing cell viability and increasing apoptosis<sup>38</sup>.

### 2.8. Amide-containing carbazole derivatives

Amide-containing carbazole derivatives **7a–d** and **8** (Figure 12) have been synthesized and their in vitro anti-proliferative activities against NPC-TW01 (nasopharyngeal carcinoma), NCI-H661 (lung carcinoma) and Jurkat (leukaemia) cell lines were evaluated. All carbazole derivatives were inactive or weakly active, with  $IC_{50}$  values ranging from 11.09 to 42.77  $\mu$ M<sup>39</sup>.

**Table 1.** Overview of the major patents since 2012 related to carbazole derivatives as anti-cancer agents.

Patent number	Year of publication	Inventors	Title	Structure
EP2403855 B1	2013	Ahmed et al.	Hybrids of carbazole-bound pyrrolo [2,1-c][1,4] benzodiazepine as potential anti-cancer agents and their method of preparation <sup>19</sup>	
US8815840 B2	2014	Purandare et al.	Carbazole and carboline kinase inhibitors <sup>20</sup>	
WO2012059232 A1	2012	Demotz S et al.	Carbazole and carboline derivatives, their preparation and therapeutic applications thereof <sup>21</sup>	
US20120184590 A1	2012	Rawjewski et al.	Formulations of indole-3-carbinol derived anti-tumour agents with increased oral bioavailability <sup>22</sup>	
WO2013121385 A1	2013	Rault et al.	Use of carbazole-phenone derivatives for treating cancer <sup>23</sup>	
WO2014134232 A1	2014	Poss et al.	Carbazole compounds useful as bromodomain inhibitors <sup>24</sup>	
US20160024083 A1	2016	Gurova et al.	Compounds and methods for treating cancers <sup>25</sup>	
US20140303224 A1	2014	Tucker et al.	Carbazole compounds and therapeutic uses of the compounds <sup>26</sup>	

(continued)



Table 1. Continued.

Patent number	Year of publication	Inventors	Title	Structure
US2017158636 A1	2017	James et al.	Functionalized and substituted carbazoles as anti-cancer agents <sup>27</sup>	
US2017166526 A1	2017	Narayanan et al.	Selective androgen receptor degrader (SARD) ligands and methods of use thereof <sup>28</sup>	

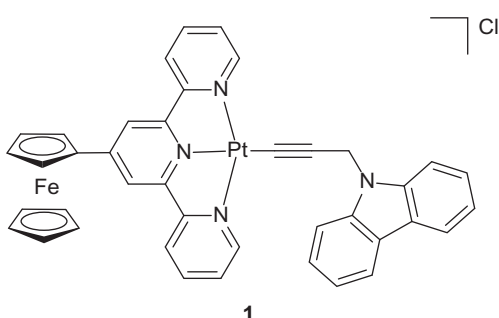


Figure 5. Chemical structure of a ferrocenyl-terpyridine platinum(II) complex 1.

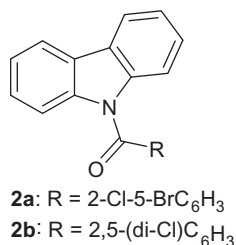
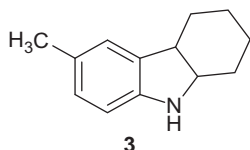
Figure 6. Chemical structures of *N*-acyl carbazole derivatives 2a and 2b.

Figure 7. Chemical structure of tetrahydrocarbazole 3.

### 2.9. Murrayafoline-A

Isolated from *Murraya euchrestifolia* (Rutaceae), 13 carbazole alkaloids were evaluated against HL-60 leukaemia cell line. Murrayafoline A (Figure 13) displayed a significant interaction with the caspase-9/caspase-3 pathway, leading to the cellular apoptosis<sup>40</sup>.

### 2.10. Clauszoline-I

Extracted from *Clausena vestita* Tao, clauszoline-I (Figure 14) showed effective ability to induce the cell cycle arrest in the S

and G2/M phases. The mechanism is linked with the inhibition of the phosphorylation of the Ser-643 of the protein kinase C delta (PKC $\delta$ ). PKC $\delta$  is a prototypical class of serine/threonine kinases, and implicated in nearly all stages of cancer<sup>41</sup>, and the induction of the cell cycle arrest. Clauszoline-I displayed a growth inhibitory activity against four cancer cell lines (cervical carcinoma, glioblastoma, nasopharyngeal carcinoma, hormone-independent breast cancer), with IC<sub>50</sub> values in the micromolar range (13.3–71.6  $\mu$ M)<sup>42</sup>.

### 2.11. 6-Methoxy-1,4-dimethyl-N-alkylcarbazole derivatives

Synthesized *N*-alkylcarbazole derivatives **9a–c** (Figure 15) showed interesting anti-proliferative activities. Their selective properties allowed the suppression of STAT3 phosphorylation, which led to the decrease of its mediated transcription with inhibition of 50, 90 and 95%, respectively<sup>43</sup>.

### 2.12. Excavatine A

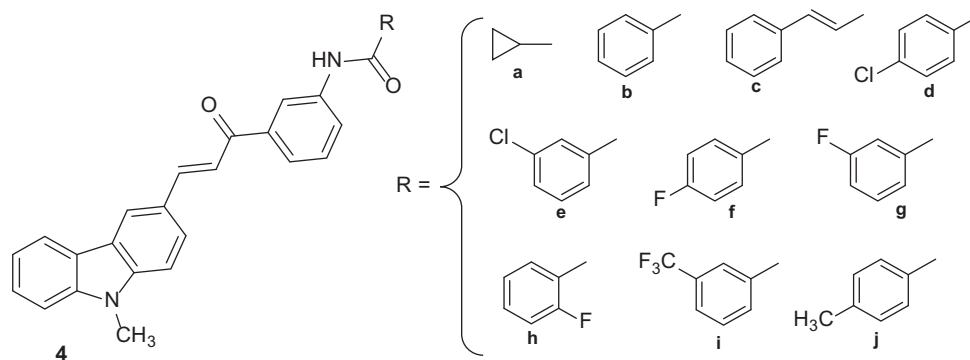
A carbazole alkaloid, excavatine A (Figure 16), was isolated from the stems and leaves of *Clausena excavata* BURM. f. (Rutaceae) and its cytotoxic activities against A549 lung carcinoma and HeLa cervix adenocarcinoma cell lines were assessed, showing IC<sub>50</sub> values of 17.77 and 6.47  $\mu$ M, respectively<sup>44</sup>.

### 2.13. Clausenawalline F

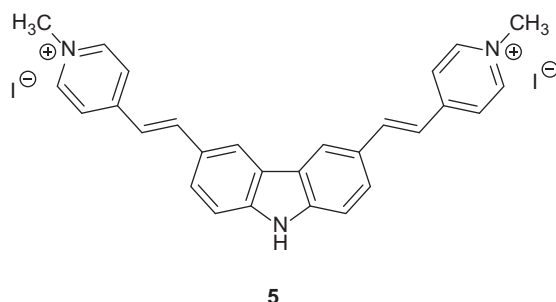
Twenty-two compounds were isolated from the roots of *Clausena wallichii* (Rutaceae) and tested for both anti-bacterial and cytotoxicity activities. After evaluations, clausenawalline F (Figure 17) exhibited the highest cytotoxicity with IC<sub>50</sub> values of 10.2  $\mu$ M against KB, a subline of the ubiquitous keratin-forming tumour cell line, and of 4.5  $\mu$ M against NCI-H187 small-cell lung cancer cell line<sup>45</sup>.

### 2.14. 2-[(9-Ethyl-9H-carbazol-3-yl)amino]-2-oxoethyl *N,N*-disubstituted dithiocarbamate derivatives

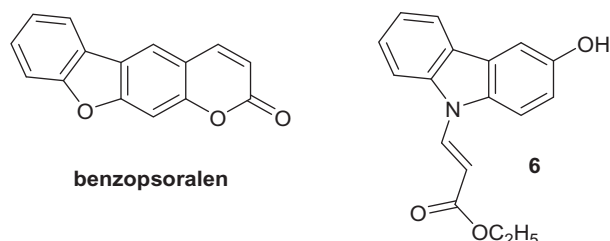
Carbazole derivatives bearing dithiocarbamate group showed cytotoxic activity on C6 glioma and A549 lung carcinoma cell lines: analysis of DNA synthesis and detection of apoptosis by flow cytometry were set up. Çiftiçi et al. reported that compound **12** (Figure 18) was the most active against C6 cell line (IC<sub>50</sub> value



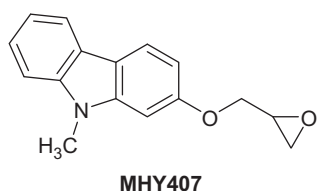
**Figure 8.** Chemical structures of N-{3-[3-(9-methyl-9H-carbazol-3-yl)-acryloyl]-phenyl}-benzamide derivatives **4a–j**.



**Figure 9.** Chemical structure of BMVC or compound **5**.



**Figure 10.** Chemical structures of benzopsoralen and (E)-ethyl-3-(3-hydroxy-9H-carbazol-9-yl)acrylate **6**.



**Figure 11.** Chemical structure of MHY407.

of 12.2  $\mu\text{M}$ ) but showed less activity against A549 ( $\text{IC}_{50}$  value of 84.7  $\mu\text{M}$ ). Compounds **10** and **11** (Figure 18) showed interesting activity against C6 cell line, with  $\text{IC}_{50}$  values of 62.7 and 49.9  $\mu\text{M}$ , respectively<sup>7,46</sup>.

### 2.15. EHOp-016

EHOp-016 was serendipitously discovered to be the most potent Rac1 inhibitor. The small GTPase Rac1 is a member of the Ras superfamily of GTPases and has been implicated in the regulation of cellular migration and invasion in breast cancer cells. EHOp-016 reduced metastatic cancer cell viability at a concentration inferior to 5  $\mu\text{M}$ . Additionally, its anti-cancer activity (tumour growth and metastasis) was demonstrated in vivo by using a mouse model of

breast cancer. The carbazole group contributed to Rac1 inhibitory activity and then new compounds **13a** and **13b** (Figure 19) were designed and synthesized. The most potent Rac1 inhibitor was **13b**, which inhibited by 55% at a concentration of 250 nM and was four times more potent inhibitor of Rac1 than EHOp-016 with reduced cellular toxicity<sup>47</sup>.

### 2.16. Carbazole-3,6-diamine derivatives

Carbazole derivatives bearing diamine groups presented a new potential for telomerase inhibition. Using three different docking programs (CDOCKER, Ligandfit, Autodock) and interaction analysis demonstrated that compounds **14a** and **14b** (Figure 20) had the best telomerase inhibition activity more interestingly with the introduction of a pyrazole ring<sup>48</sup>.

### 2.17. Carbazole sulfonamide derivatives

The carbazole sulfonamide IG-105<sup>49</sup> (Figure 21) was described as a potent anti-mitotic agent that inhibited microtubule assembly through specific interactions within the tubulin structure. The introduction of a hydroxyl group (7-OH) on the carbazole-ring increased the solubility and provided a new derivative named SL-3-19 (Figure 21). This compound was active against HepG2 liver cancer ( $\text{IC}_{50}$  = 12 nM) and MCF-7 ( $\text{IC}_{50}$  = 14 nM) breast cancer cell lines. The  $\text{IC}_{50}$  of the positive controls podophyllotoxin and combretastatin CA-4<sup>49</sup> against HepG2 were 3 and 2 nM, respectively, and against MCF-7 ( $\text{IC}_{50}$  = 20 and 5 nM, respectively). Niu et al. described further investigations on the anti-oesophageal squamous cell carcinoma (ESCC) activity and mechanisms of SL-3-19 in vitro and in vivo. Mechanistically, SL-3-19 inhibited ESCC cell growth by inducing cell apoptosis and arresting the cell cycle at G2/M phase in a dose-dependent manner. In addition to microtubule assembly inhibition, this compound showed a significant disruption of the vascular structure by obstructing the formation of capillary-like tubes in vitro and the growth of ESCC xenografts and microvessel density in vivo<sup>50</sup>.

### 2.18. Trimethoxybenzamide and trimethoxyphenylurea

Two 1,4-dimethylcarbazole derivatives (Figure 22), trimethoxybenzamide **15** and trimethoxyphenylurea **16**, were active against both HL60 (acute promyelocytic leukaemia) and KB (a subline of HeLa) cell lines. These compounds are potent cell proliferation inhibitors, especially **15** that showed the best activities with  $\text{IC}_{50}$  values of 5.3  $\mu\text{M}$  against HL60 cells and 6.7  $\mu\text{M}$  against KB cells. The anti-proliferative activity was correlated with the inhibition of tubulin polymerization, which ranged from 20 to 50% inhibition<sup>51</sup>.



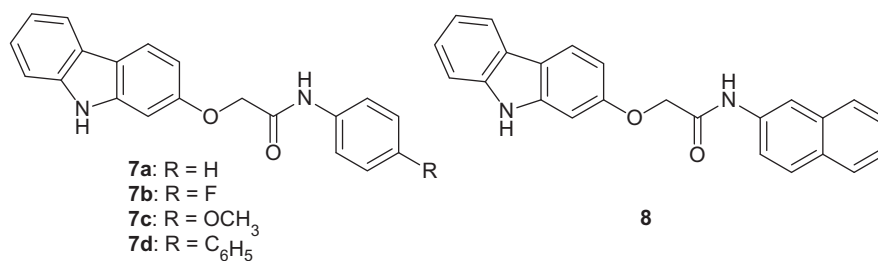
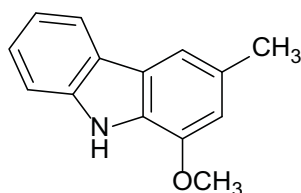
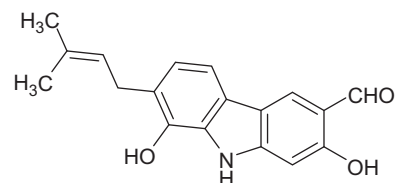


Figure 12. Chemical structures of amide containing carbazole derivatives 7a–d and 8.



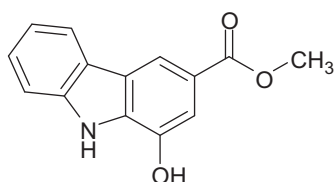
**murrayafoline A**

Figure 13. Chemical structure of murrayafoline A.



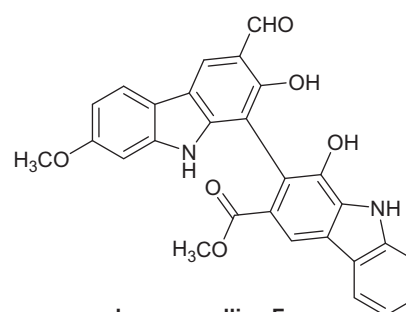
**excavatine A**

Figure 16. Chemical structure of excavatine A.



**clauszoline-I**

Figure 14. Chemical structure of clauszoline-I.



**clausenawalline F**

Figure 17. Chemical structure of clausenawalline F.

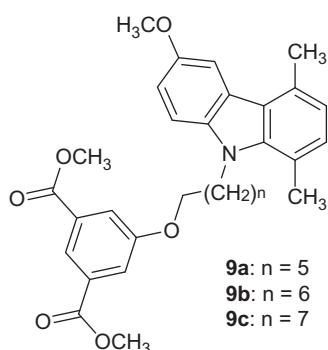


Figure 15. Chemical structures of N-alkylcarbazole derivatives 9a–c.

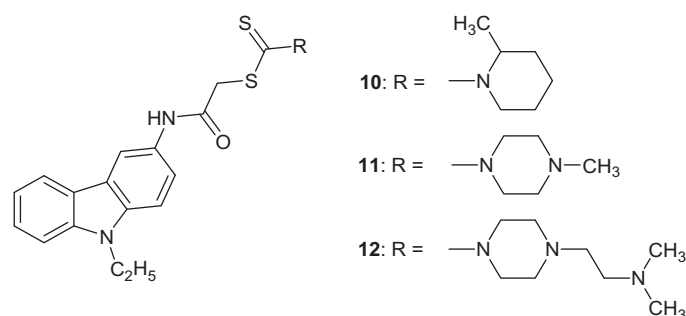


Figure 18. Chemical structures of compounds 10, 11 and 12.

## 2.19. Guanidinocarbazoles

Several alkylguanidines derived from carbazole were prepared and tested for their anti-cancer activity. Three compounds **17a–c** (Figure 23) were tested at  $10^{-5}$  M against KB and HeLa cell lines, and the best IC<sub>50</sub> values (3.1, 3.5 and 4  $\mu$ M, respectively) against HL60 acute promyelocytic leukaemia cell line. Compound **17a**, which was found to be the most active, also demonstrated a high inhibition at  $10^{-5}$  M against MCF-7, HCT116, PC3 (prostate cancer) and MRC5 (human fatal lung fibroblast) cell lines. Furthermore, fluorescence measurements were carried out and showed that **17a** had some DNA binding properties<sup>52</sup>, which was described as a cycle-dependent cytotoxic activity.

In summary, various pharmacomodulations in the series of tricyclic carbazoles were performed to obtain new anti-tumour

compounds and to investigate the structure–activity relationships. Several substituents were essentially introduced on the nitrogen atom of the indole moiety or on the benzene moiety. Figure 24 shows the substituents that have contributed to the improvement of the anti-tumour activity of the compounds described in this section.

## 3. Tetracyclic fused carbazoles

### 3.1. Tetracyclic carbazoles containing a 5-membered ring

#### 3.1.1. Cyclopenta[c]carbazoles

A synthesized series of cyclopenta[c]carbazoles were investigated as a p53 activator using two cell lines, HT1080 (lung fibrosarcoma)

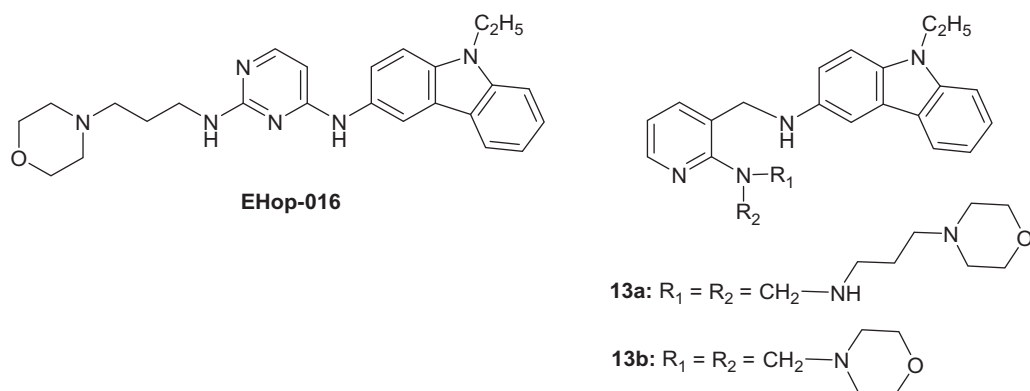


Figure 19. Chemical structures of EHop-016 and compounds 13a and 13b.

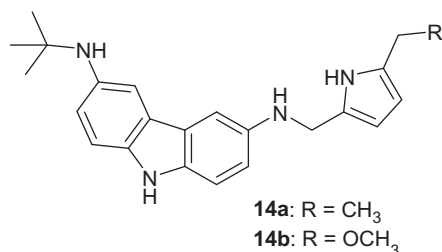


Figure 20. Chemical structures of carbazole-3,6-diamine derivatives 14a and 14b.

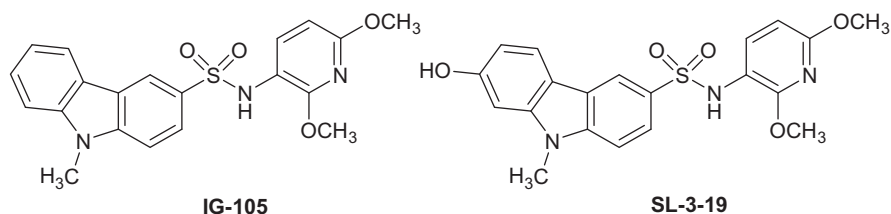


Figure 21. Chemical structures of compounds IG-105 and SL-3-19.

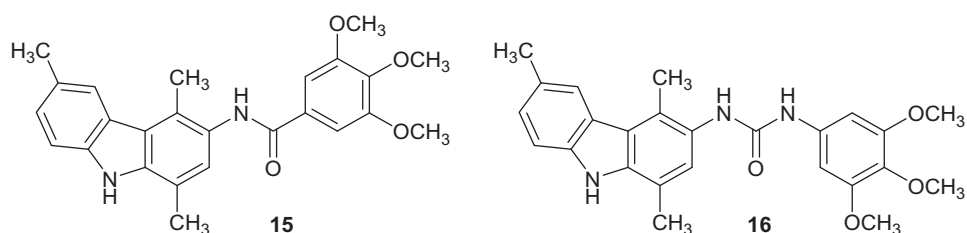


Figure 22. Chemical structures of 1,4-dimethylcarbazole derivatives 15 and 16.

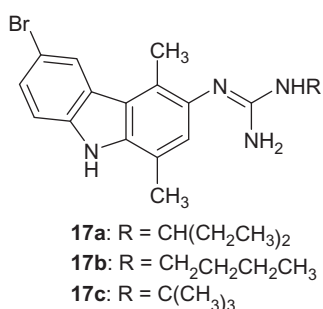


Figure 23. Chemical structures of guanidinocarbazole compounds 17a–c.

and RCC45 (renal cell carcinoma) cell lines. The highest activity ( $\text{EC}_{50}$  value of  $0.08\ \mu\text{M}$ ) was observed with compound **18** (Figure 25) having an acetyl group at C6 and N9 substituted with (1-methylpyrrolidin-2-yl)ethyl<sup>53</sup>.

### 3.1.2. Furanocarbazoles

Fourteen compounds, including mafaicheenamines D and E, were isolated from the roots of *Clausena lansium* (Rutaceae) and evaluated against KB, MCF-7 and NCI-H187 cell lines. All compounds were non-cytotoxic against the tested cell lines, except mafaicheenamine E with a methoxy group at C<sub>1</sub> and bearing a substituted furanone ring on C<sub>2</sub>–C<sub>3</sub> (Figure 26) which exhibited

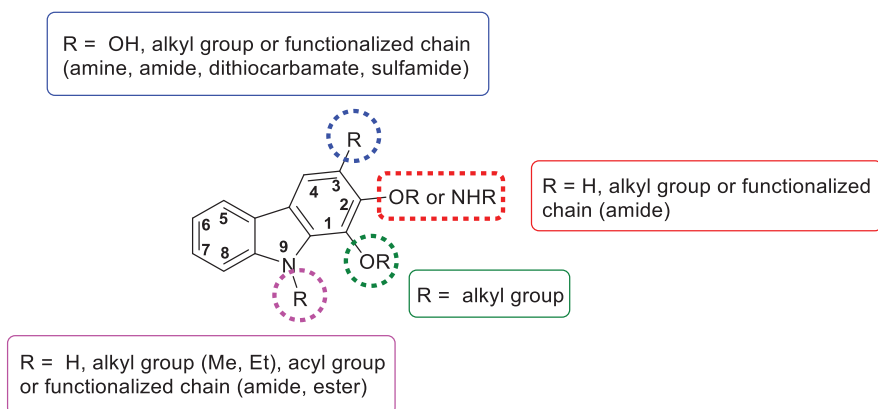


Figure 24. SAR of tricyclic carbazoles.

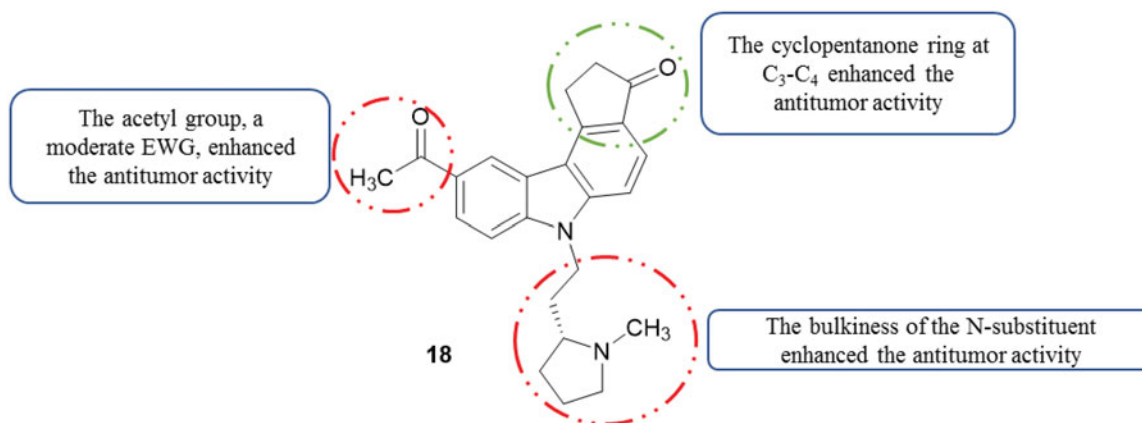


Figure 25. Chemical structure of cyclopenta[c]carbazole 18.

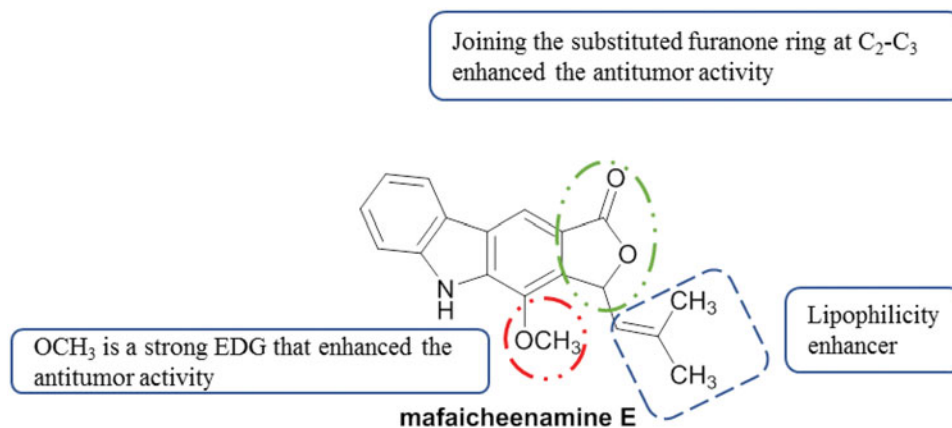


Figure 26. Chemical structure of mafaicheenamine E.

cytotoxicity against MCF-7 cell line with an IC<sub>50</sub> value of 10.1  $\mu\text{M}$ <sup>54</sup>.

### 3.1.3. Pyrrolocarbazoles

Santio et al.<sup>55</sup> first described the activity of 1,10-dihydropyrrolo[2,3-*a*]carbazole-3-carbaldehyde (DHPCC-9, compound **19a**, Figure 27) as a potent and selective inhibitor for all Pim family members. Novel *N*-10-substituted pyrrolo[2,3-*a*]carbazole-3-carbaldehyde derivatives<sup>56</sup> were synthesized and evaluated as Pim kinase inhibitors<sup>57,58</sup>. All cited compounds in Figure 27 showed

inhibitory activity of Pim kinases, especially Pim-1 and Pim-3 with IC<sub>50</sub> comprised between 46 and 490 nM. In vitro anti-proliferative activity of compounds **19f**, **19g** and **19h** was also evaluated using primary human fibroblasts and three human solid cancer cell lines (PA1, PC3 and DU145). These three compounds presented anti-proliferative activities in a micromolar range. Among them, **19h** was the most active compound (0.486 < IC<sub>50</sub> < 0.96  $\mu\text{M}$ )<sup>7</sup>.

Derivatives of *N*<sub>1</sub>-*N*<sub>10</sub>-bridged pyrrolo[2,3-*a*]carbazole-3-carbaldehyde<sup>7</sup> showed interesting inhibitory properties, especially with compounds **20a** and **20b** (Figure 28). Both molecules were presented as Pim-1 and Pim-3 inhibitors (IC<sub>50</sub> from 0.009 to 0.05  $\mu\text{M}$ ).

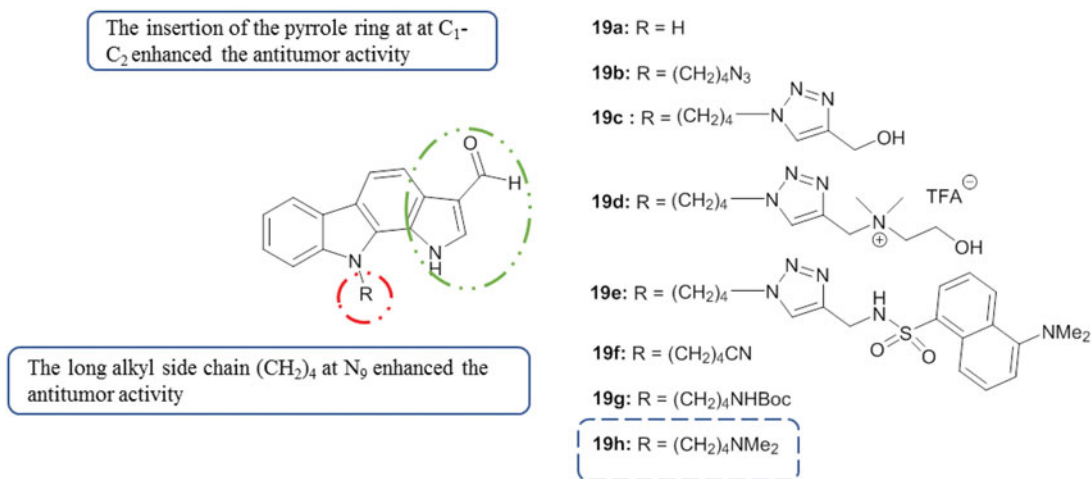


Figure 27. Chemical structures of *N*-10-substituted pyrrolo[2,3-*a*]carbazole derivatives 19a–g.

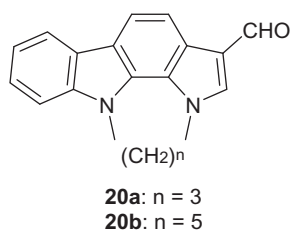


Figure 28. Chemical structures of *N*<sub>1</sub>,*N*<sub>10</sub>-bridged pyrrolo[2,3-*a*]carbazole-3-carbaldehydes 20a and 20b.

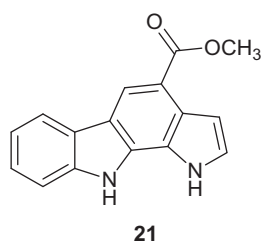


Figure 29. Chemical structure of pyrrolo[2,3-*a*]carbazole 21.

In parallel, compounds **20a** and **20b** with a longer alkyl N<sub>1</sub>–N<sub>10</sub> bridge exhibited apoptosis-inducing activity toward IPC-81 (acute myeloid leukaemia) cells, but not toward normal fibroblasts<sup>59</sup>.

Other pyrrolo[2,3-*a*]carbazole derivatives with substituent on position 4 were synthesized and their biological activities were evaluated as Pim kinase inhibitors and in vitro anti-proliferative agents. Compound **21** (Figure 29), bearing a methoxycarbonyl group at the 4-position, was found to be active, especially on Pim-3 kinase with IC<sub>50</sub> around 0.5 μM. The latter also showed anti-proliferative activities on fibroblasts (IC<sub>50</sub> = 8 μM) and on PC3 cells (IC<sub>50</sub> around 6 μM)<sup>60</sup>.

Natural C-glycosyl pyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione derivatives were tested as Checkpoint kinase 1 (Chk1) inhibitors. Compounds **22** and **23** (Figure 30) substituted at the C<sub>1</sub> with a glycosyl group and at C<sub>6</sub> with a hydroxyl group were the most active compounds among this series and exhibited IC<sub>50</sub> values from 0.5 to 9.5 μM<sup>61</sup>.

### 3.1.4. Pyrazolocarbazoles

A series of pyrazolocarbazoles [3,4-*c*] and [4,3-*c*] carbazoles was synthesized and tested. Among 3,6-dihydropyrazolo[3,4-*c*]carbazoles, compound **24** (Figure 31) demonstrated to potently inhibit

Pim-1 and Pim-3 kinases (IC<sub>50</sub> from 0.04 to 0.1 μM) and also to be active against proliferative activities of prostatic cancer cells PC3 (IC<sub>50</sub> was around 3 μM). A 1,6-dihydropyrazolo[4,3-*c*]carbazole, compound **25** (Figure 31), showed the best Pim kinase inhibitory potency toward Pim-3 (IC<sub>50</sub> around 0.09 μM)<sup>62</sup>.

### 3.1.5. Isoxazolocarbazoles

A series of carbazole analogues comprising pyrido, isoxazolo, pyrimido and pyrazolo templates were synthesized and evaluated for their cytotoxicity against AGS (gastric cancer) and HeLa cell lines. Among the tested derivatives, 3-(3',4'-diethoxyphenyl)-9-methyl-4,5-dihydro-10*H*-isoxazolo[3,4-*a*]carbazole (compound **26**, Figure 32) stood out with an IC<sub>50</sub> value of 0.37 μM against HeLa cells, which was 11 times fold better than the standard ellipticine. All the compounds exhibited a strong in vitro and selective cytotoxicity against HeLa and moderate activities against AGS cell line<sup>63</sup> (for compound **26**, IC<sub>50</sub> of 15.12 μM).

Briefly, when comparing the mentioned tetracyclic carbazoles containing a five-membered ring, SAR study can be correlated with substitutions at the carbazole ring (Figure 33).

## 3.2. Tetracyclic carbazoles containing a six-membered ring

### 3.2.1. Benzocarbazoles

Alectinib/CH5424802 (compound **27a**, Figures 2 and 34) is the second generation ALK inhibitor bearing a 5*H*-benzo[*b*]carbazol-11(6*H*)-one structural scaffold which presented a high selective ALK inhibition at a nanomolar scale (IC<sub>50</sub> value of 1.9 nM)<sup>64</sup>. Additionally, it is a potent anti-proliferative compound against KARPAS-299 cell line (human T cell lymphoma) carrying the nucleophosmin (*NPM*)-*ALK* fusion gene with an IC<sub>50</sub> value of 3.0 nM. In vivo studies in mice, using *ALK* fusion gene-positive NSCLC xenograft model, showed that orally administrated compound **27a** significantly regressed tumours. Currently, this compound is being evaluated in phase I/II clinical trials for the treatment of *ALK*-positive NSCLC<sup>65</sup>.

Compared to the first generation non-carbazole derivative crizotinib (Figure 35), in vitro and in vivo studies showed that alectinib was more potent and selective against wild and mutant *ALK*. Kodama et al.<sup>66</sup> showed that the inoculation of alectinib reduced the tumour size and avoided its regrowth. As previously mentioned, alectinib received in 2015 approvals by FDA and EMA for anti-cancer use.

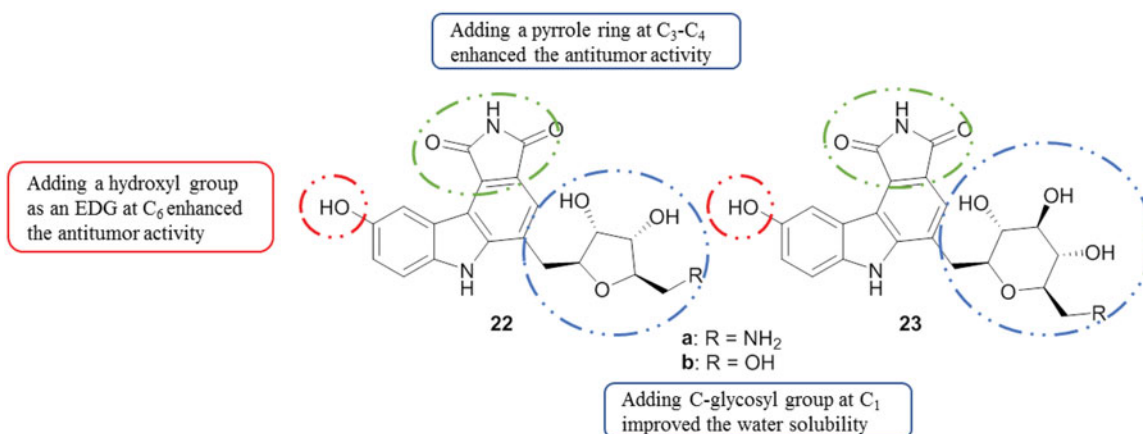


Figure 30. Chemical structures of Chk1 inhibitors 22 and 23.

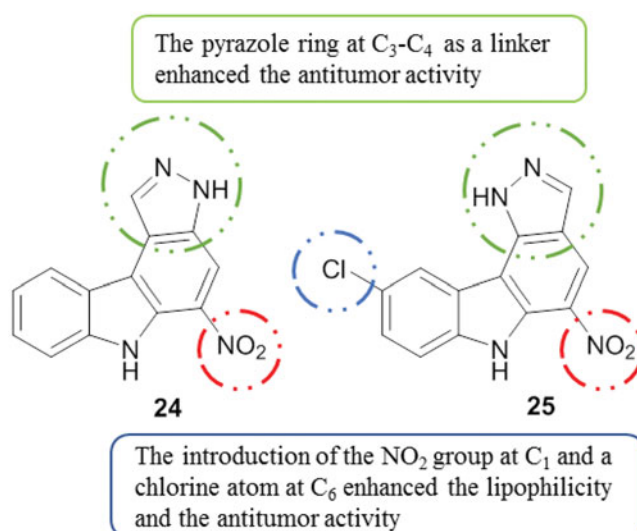


Figure 31. Chemical structures of pyrazolo[3,4-c]carbazole 24 and pyrazolo[4,3-c]carbazole 25.

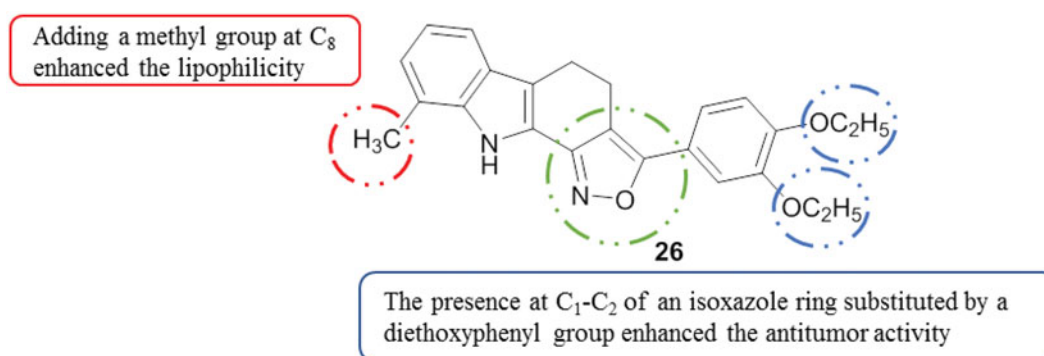


Figure 32. Chemical structure of the isoxazolocarbazole derivative 26.

A series of 2-(4-aminobenzosulfonyl)-5*H*-benzo[*b*]carbazole-6,11-dione derivatives has been synthesized. In vitro anti-proliferative activity was performed against SiHa (cervical carcinoma) cell lines. Compounds **28**, **29a,b** (Figure 36) exhibited a good cytotoxicity with IC<sub>50</sub> values of 52.2, 53.8 and 33.5 μM, respectively. The interaction between all compounds and HDAC8 was also carried out by performing molecular docking studies with the use of the GLIDE program<sup>67</sup>.

### 3.2.2. Pyranocarbazoles

Girinimbine (Figure 37), a carbazole alkaloid isolated from the stem bark of *M. koenigii*, had a strong anti-tumour promoting activity. The expression of the Epstein-Barr Virus Early Antigen (EA-EBV) in Raji cells was inhibited by more than 90% when tested at 16 μg/mL (50% inhibition at 22.8 μM). The compound did not alter Raji cell's viability due to very low cytotoxicity. Girinimbine showed strong anti-oxidant properties comparable to α-tocopherol



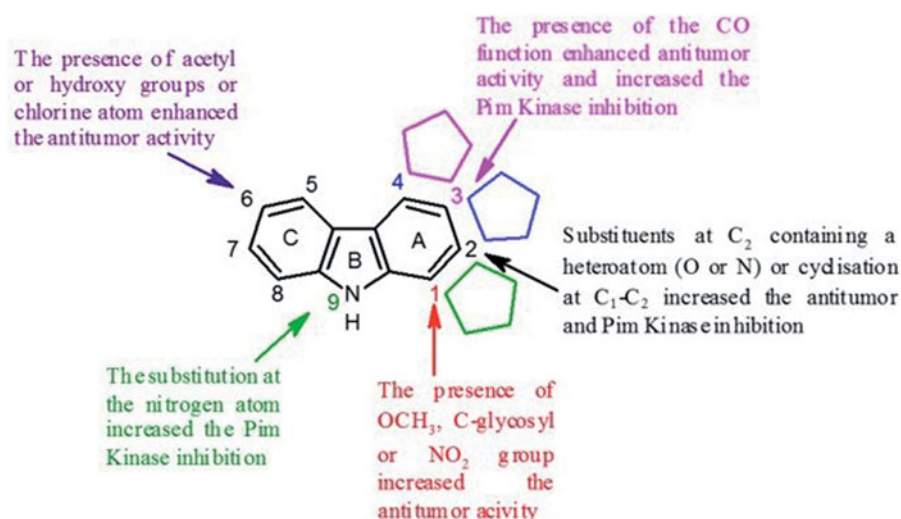


Figure 33. SAR study of tetracyclic carbazoles containing a 5-membered ring.

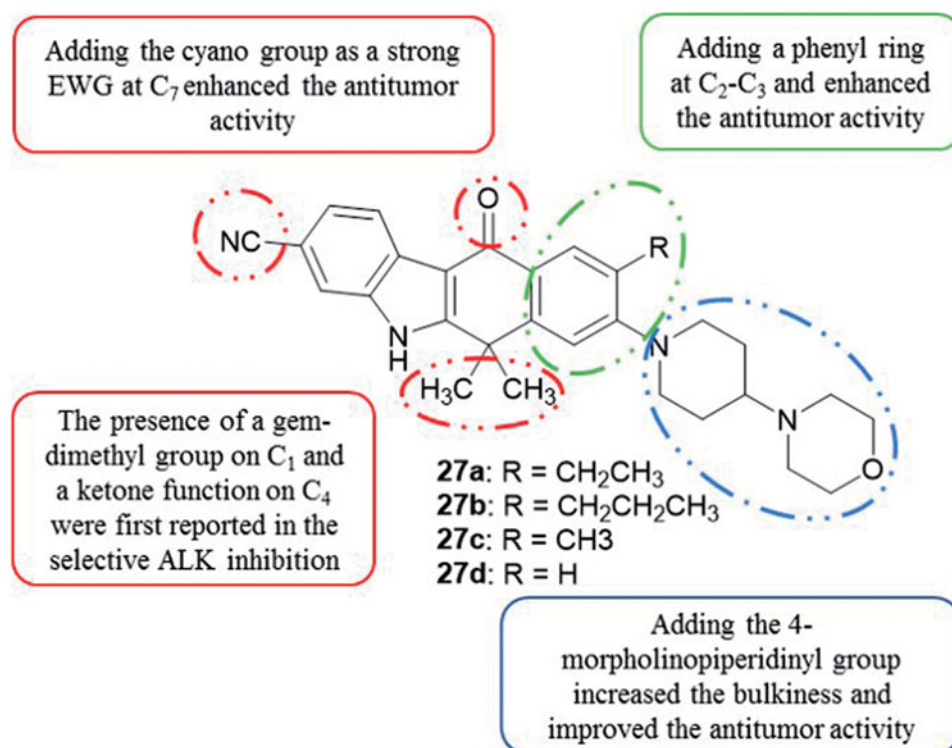


Figure 34. Chemical structures of compounds 27a–d as ALK inhibitors.

(vitamin E) and inhibited the superoxide generation in the 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced differentiated HL-60 cells<sup>68</sup>.

A series of pyrano[3,2-c]carbazole derivatives **30a–d** (Figure 38) showed interesting anti-proliferative activity on different cancer cell lines such as MDA-MB-231, K562, A549 and HeLa with IC<sub>50</sub> values ranging from 0.43 to 8.05  $\mu$ M. The MTT cell proliferation and tubulin polymerization assays demonstrated that these compounds induced a G2/M arrest of the cell cycle by inhibiting tubulin and disrupting the microtubule network. The caspase-3 assay demonstrated that the cell death occurred by apoptosis<sup>69</sup>.

Mahanine (3,5-dimethyl-3-(4-methylpent-3-en-1-yl)-3,11-dihydro-pyrano[3,2-a]carbazol-9-ol, Figure 39) has been tested in several studies as a single compound or in combination for anti-cancer therapy, for example HCT116 (IC<sub>50</sub>=25.5  $\mu$ M), HeLa

(IC<sub>50</sub>=24.3  $\mu$ M) and AGS (IC<sub>50</sub>=33.8  $\mu$ M) cancer cell lines were employed. It showed a growth inhibitory effect with IC<sub>50</sub> in the micromolar range (from 12.6 to 33.8  $\mu$ M). Mahanine is also a DNA intercalative cytotoxic molecule, which presented anti-oxidant capacities. On human cancer cell lines, it indicated excellent radical scavenging of the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•, 9.2  $\mu$ M), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid radical (ABTS•+, 6837.5  $\mu$ mol Trolox/g), OH• (12.0  $\mu$ M) and nitric oxide radical (NO•, 7.8  $\mu$ M)]. Mahanine showed as well alpha-glucosidase inhibitory activity with IC<sub>50</sub> value of 21.4  $\mu$ M<sup>70</sup>.

Das et al.<sup>71</sup> reported that the combination of mahanine with 5-fluorouracil enhanced the reactive oxygen species (ROS) production, increased the activation of tumour suppressor proteins and suppressed chemo-migration. In another study<sup>72</sup>, the same authors reported that when associated to cisplatin, mahanine



could overcome cisplatin-toxicity and drug resistance. Mahanine synergically improved the apoptosis induced by cisplatin in cervical cancer cells and inhibited migration property. The combination at molar ratio 1:4 of cisplatin: mahanine showed a growth inhibitory effect on HeLa and SiHA cell lines ( $IC_{50}$  of 1.6–1.8  $\mu$ M, respectively). This effect on cell inhibition was 10 times higher to the inhibition induced only by mahanine.

### 3.2.3. Pyridocarbazoles

The commercial analogue of ellipticine, Celiptium® (Figure 1), is active against metastatic breast cancer and acts as an inhibitor of type II topoisomerase. Several studies have also reported apoptosis induction by ellipticine involving the p53 tumour suppressor protein. Prudent et al.<sup>73</sup> have recently reported a novel mechanism of action of ellipticine as a new inhibitor of the casein kinase CK2 and new analogues are currently developed.

Mori et al.<sup>74</sup> reported the synthesis of new ellipticine and pyridocarbazole derivatives and their evaluation against HeLa S-3 cell lines. Most of the compounds showed anti-tumour activity with  $IC_{50}$  values between 2.50  $\mu$ M and 60  $\mu$ M. It appeared that compound **31** (Figure 40), an ellipticinium-analogue linked to a methylnitrosourea group, showed the best anti-tumour activity with an  $IC_{50}$  value of 1.3  $\mu$ M which was two times more potent than ellipticine (2.1  $\mu$ M).

Quantitative structure activity analysis (QSAA) was carried out on olivacine and compounds **32a–i** (Figure 41) using the software TSAR to determine the structural features responsible for their

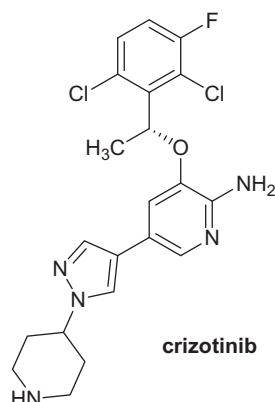


Figure 35. Chemical structures of crizotinib (Xalkori®), a second generation of ALK inhibitors.

activity<sup>75</sup>.

Hetero annulated carbazoles were designed, synthesized and their in vitro cytotoxicity was evaluated against HeLa and MCF-7 cell lines by MTT assay and compared to the standard drug ellipticine. Compound **33** (Figure 42) demonstrated 1.2-fold stronger activity than ellipticine's cytotoxic activity against HeLa. Then, molecular docking studies were carried out using CK2 as a target, in which compound **33** showed the lowest binding energy and best ligand efficiency<sup>76</sup>. SAR studies revealed that, the compound bearing the pyrimido moiety and the electron-withdrawing chlorine in the carbazole displayed excellent cytotoxic activity ( $IC_{50}$  value of 8.11  $\mu$ M) against HeLa cells.

The in vitro cytotoxicity of the pyrido[2,3-*a*]carbazoles was evaluated by SRB (sulfo-rhodamine B) assay against MCF-7, HeLa and A549 cell lines. Among these derivatives, compound **34** (Figure 43) showed the best activity with an  $IC_{50}$  of 13.42  $\mu$ M against HeLa cells (cisplatin, 13.20  $\mu$ M). All the designed compounds demonstrated a higher potency against HeLa than against the other tested cell lines<sup>77</sup>.

Pyrido[3,2-*a*]carbazole derivatives and their analogues were tested against A549 and HT29 cell lines with  $IC_{50}$  values ranging from 0.07  $\mu$ M to 4.45  $\mu$ M. For example, compound **35** (Figure 44) was active against A549 cells with an  $IC_{50}$  value of 0.07  $\mu$ M and with 0.11  $\mu$ M against HT29 cells<sup>78</sup>.

Ditercalinium is a dimer of two 7*H*-pyrido[4,3-*c*]carbazole units (Figure 45). It is a bis-interacting agent in the major groove of DNA. NMR studies and X-ray crystal structure revealed that both rings of the dimer allowed intercalation with base pairs and caused structural changes in the DNA<sup>79</sup>.

### 3.2.4. Pyrimidocarbazoles

Among pyrimidocarbazole derivatives **36a–d** (Figure 46), 2-amino-4-(3'-bromo-4'-methoxyphenyl)-8-chloro-11*H*-pyrimido[4,5-*a*]carbazole **36d** showed the best cytotoxic efficacy against both MCF-7

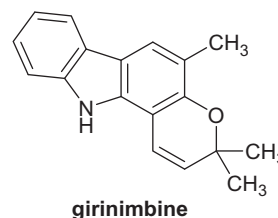


Figure 37. Chemical structure of girinimbine.

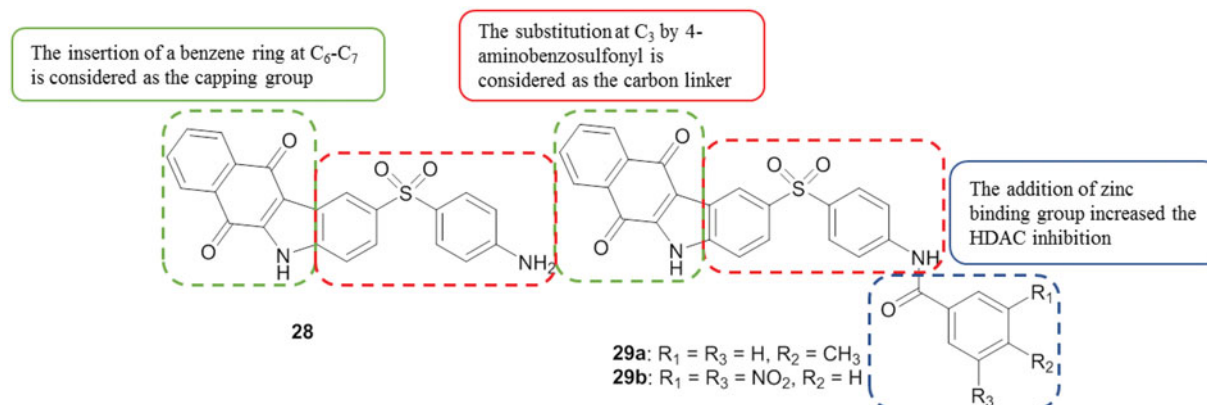


Figure 36. Chemical structures of 2-(4-amino-benzosulfonyl)-5*H*-benzo[*b*]carbazole-6,11-diones **28** and **29a,b**.

Based on SAR results, compounds having a pyran ring substituted with  $\text{NH}_2$  and an EWG presented a higher potential cytotoxicity with CN compared to  $\text{COOC}_2\text{H}_5$  group

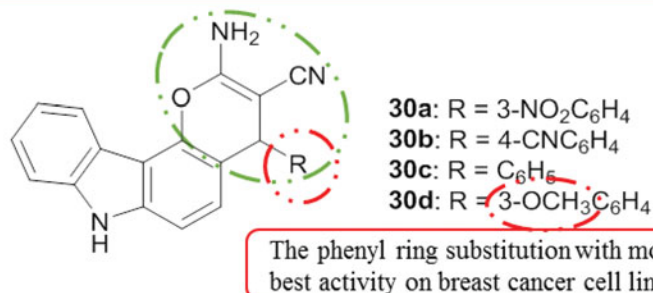


Figure 38. Chemical structures of some pyrano[3,2-c]carbazole derivatives 30a–d.

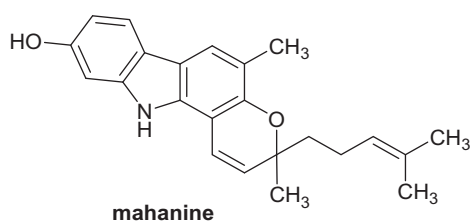


Figure 39. Chemical structure of mahanine.

A pyridine ring at  $\text{C}_2\text{--C}_3$  and the substitution of  $\text{C}_1$  with an EWG, improved the activity. The insertion of a methyl group on  $\text{C}_4$  increased the lipophilicity

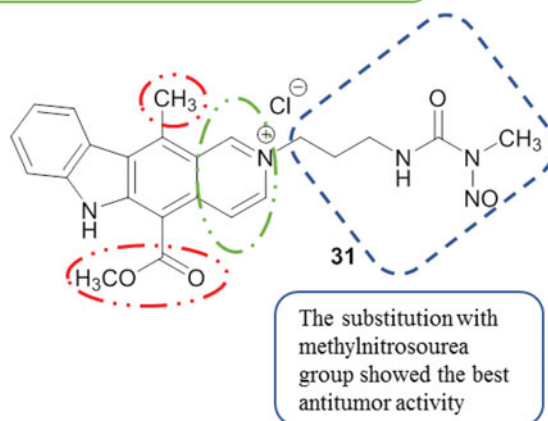


Figure 40. Chemical structure of the pyridocarbazole 31.

and A-459 cancer cell lines ( $\text{IC}_{50}$  value of 20 and 25  $\mu\text{M}$ , respectively). Compounds **36a–c** also exhibited stronger cytotoxic activity against MCF-7 cell lines<sup>80</sup>.

### 3.2.5. Oxazinocarbazoles

A set of various oxazinocarbazoles was synthesized<sup>81</sup> and their activities were studied using a CE-based assay for CK2 activity measurement, a cytotoxicity assay using IPC-81 cells. Three oxazinocarbazoles **37a–c** (Figure 47) showed CK2 inhibition with  $\text{IC}_{50}$  values of 8.7, 14.0 and 1.40  $\mu\text{M}$ , respectively. Another test using

again the IPC-81 cells was then performed, and those compounds demonstrated the ability to induce leukaemia cell death with  $\text{IC}_{50}$  values between 57 and 62  $\mu\text{M}$ <sup>81</sup>.

### 3.2.6. Miscellaneous

The cytotoxicity of three dihydro-4H-pyrido[3,2,1-jk]carbazole derivatives was evaluated against HeLa cell lines. Carbazoles **38**, **39** and **40** (Figure 48) showed moderate activity with  $\text{IC}_{50}$  values of 19.80, 17.46 and 18.76  $\mu\text{M}$ , respectively<sup>82</sup>.

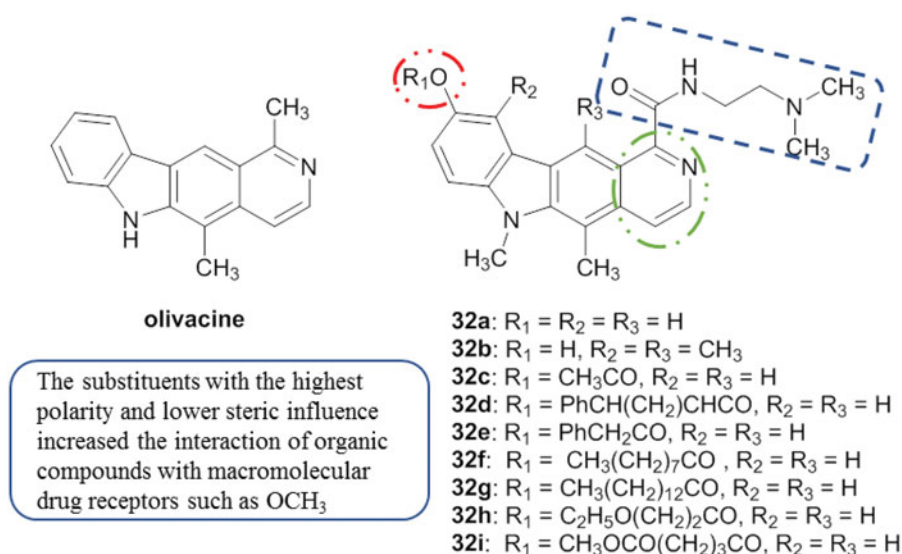


Figure 41. Chemical structures of olivacine and derivatives 32a–i used for 2D QSAR analysis.

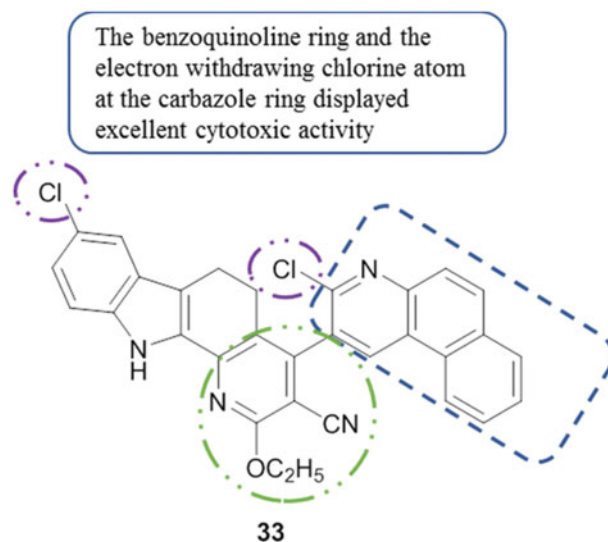


Figure 42. Chemical structure of heteroannulated carbazole compound 33.

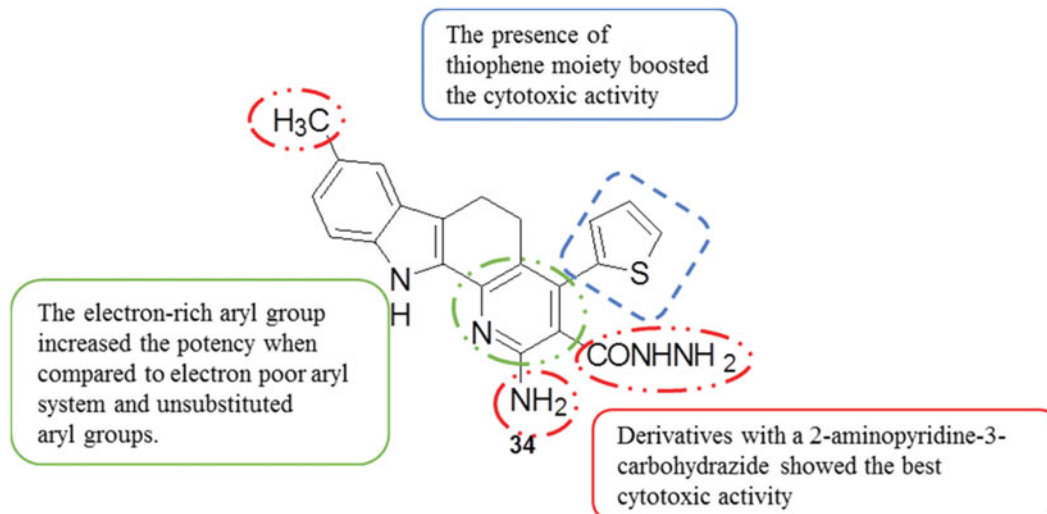


Figure 43. Chemical structure of the pyridocarbazole 34.

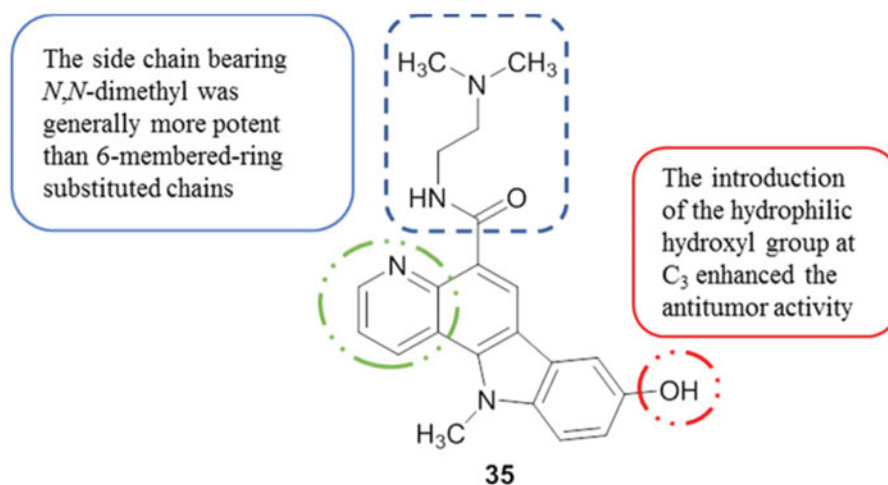


Figure 44. Chemical structure of pyridocarbazole 35.

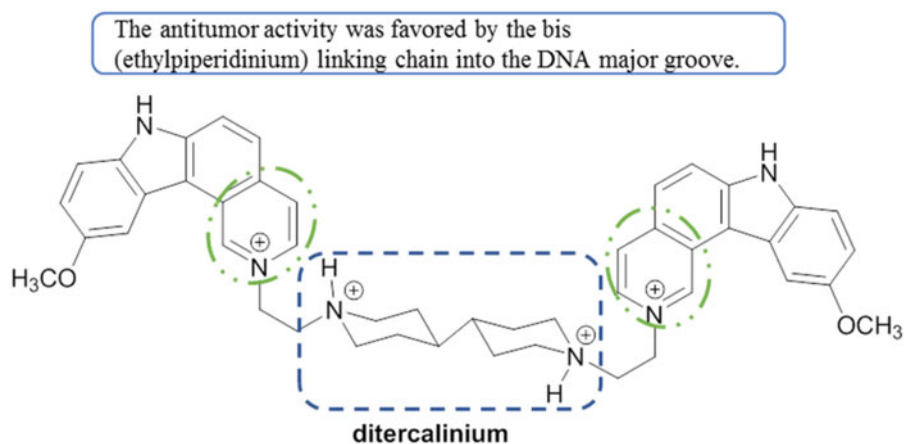
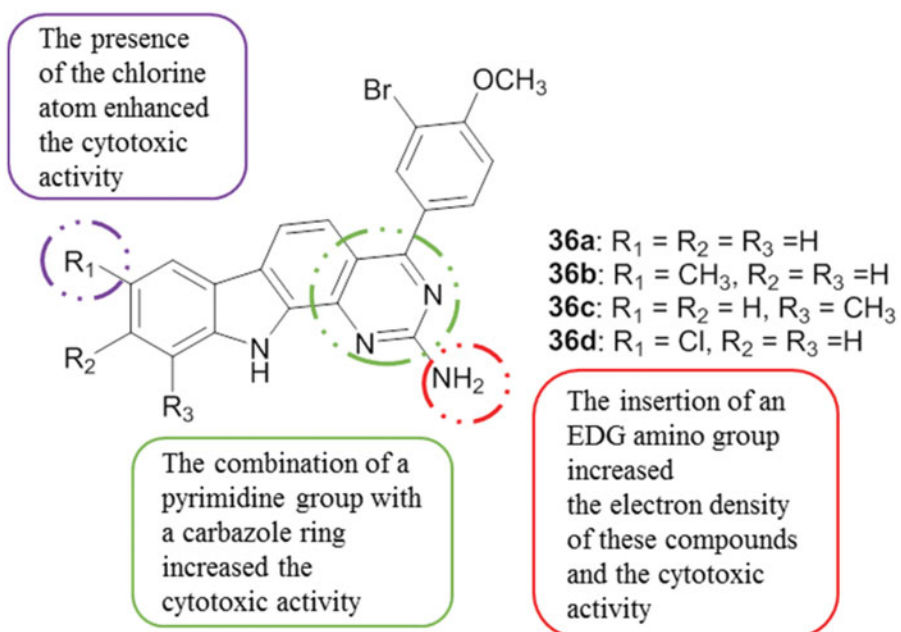


Figure 45. Chemical structure of ditercalinium.

Figure 46. Chemical structures of pyrimido[4,5-*a*]carbazole derivatives 36a–d.

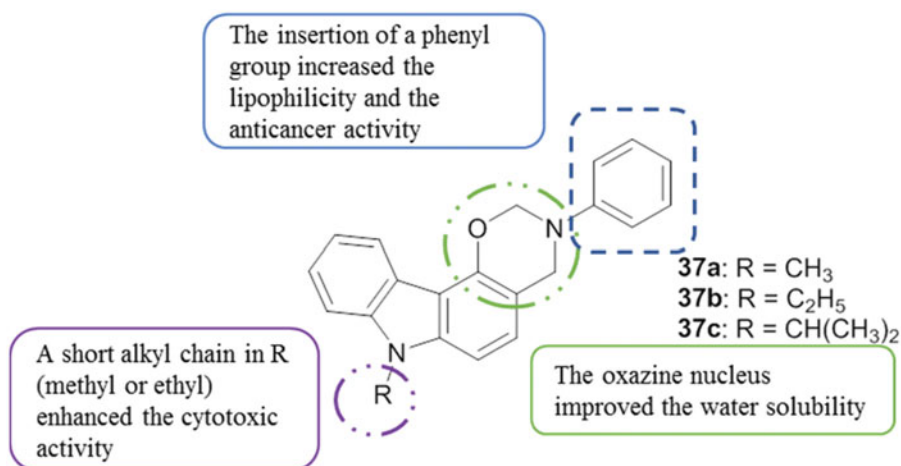


Figure 47. Chemical structures of oxazinocarbazole derivatives 37a–c.

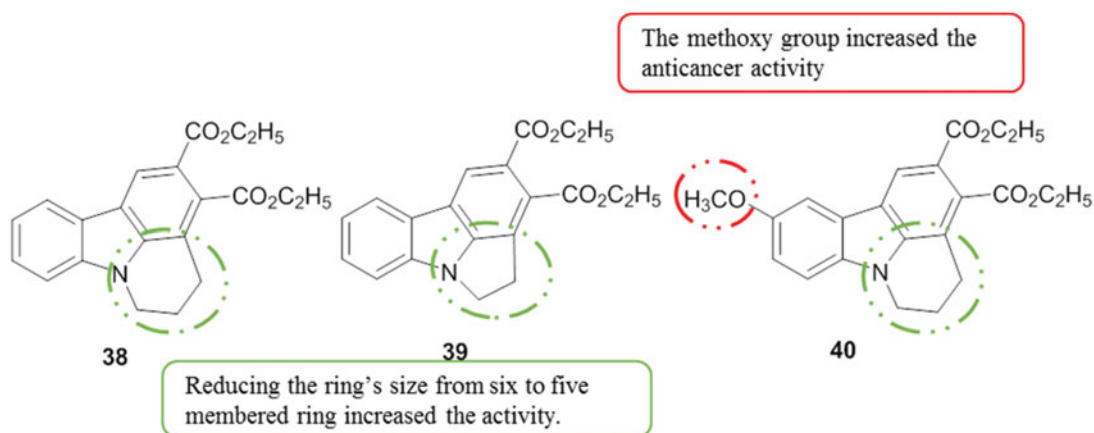


Figure 48. Chemical structures of the tetracyclic carbazoles 38, 39, and 40.

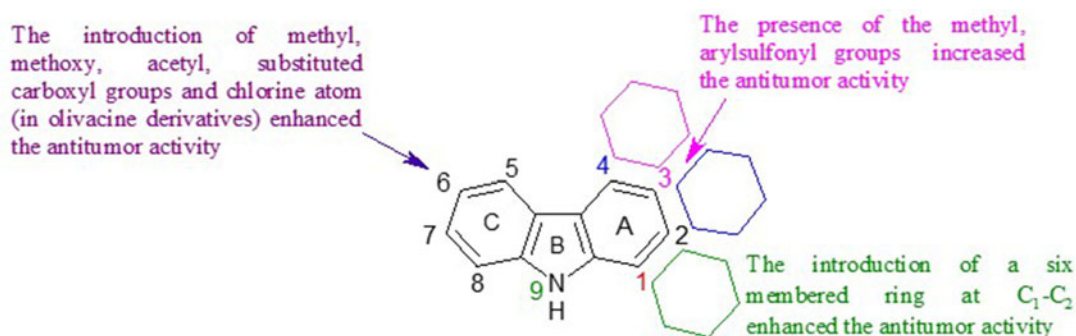


Figure 49. SAR of tetracyclic carbazoles containing a 6-membered ring.

Briefly, when comparing the mentioned tetracyclic carbazoles containing a six-membered ring SAR can be correlated with substitutions at the carbazole ring (Figure 49).

### 3.3. Tetracyclic carbazoles containing a 7-membered ring

#### 3.3.1. 1,4-Thiazepan-3-ones fused carbazoles

Several 1,4-thiazepine derivatives fused with carbazole skeleton underwent *in vitro* cytotoxic studies. Compounds **41a–f**

(Figure 50) showed selective cytotoxicity towards HCT 116 cell lines with inhibition rates of 51.57–62% at 1 mg/mL<sup>83</sup>.

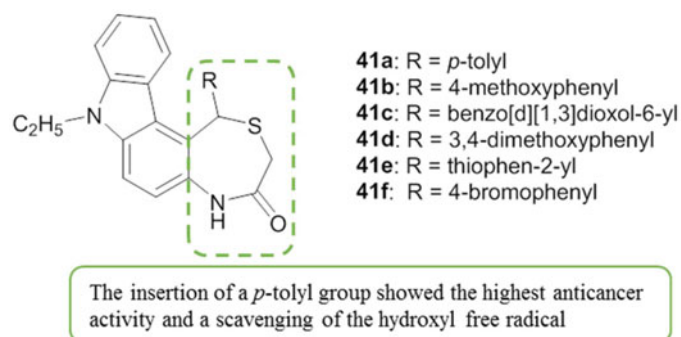
## 4. Pentacyclic fused carbazoles

### 4.1. Pentacyclic fused carbazoles containing two 5-membered rings

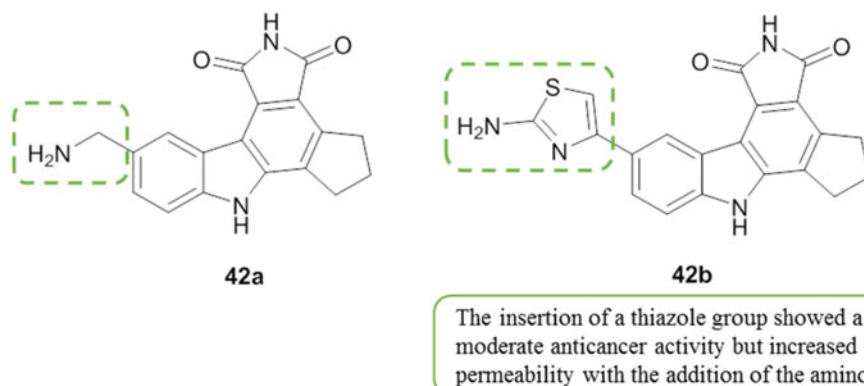
A series of 3-substituted-pyrrolocarbazole analogues (3-aryl-derived pyrrolocarbazoles) was synthesized and evaluated as



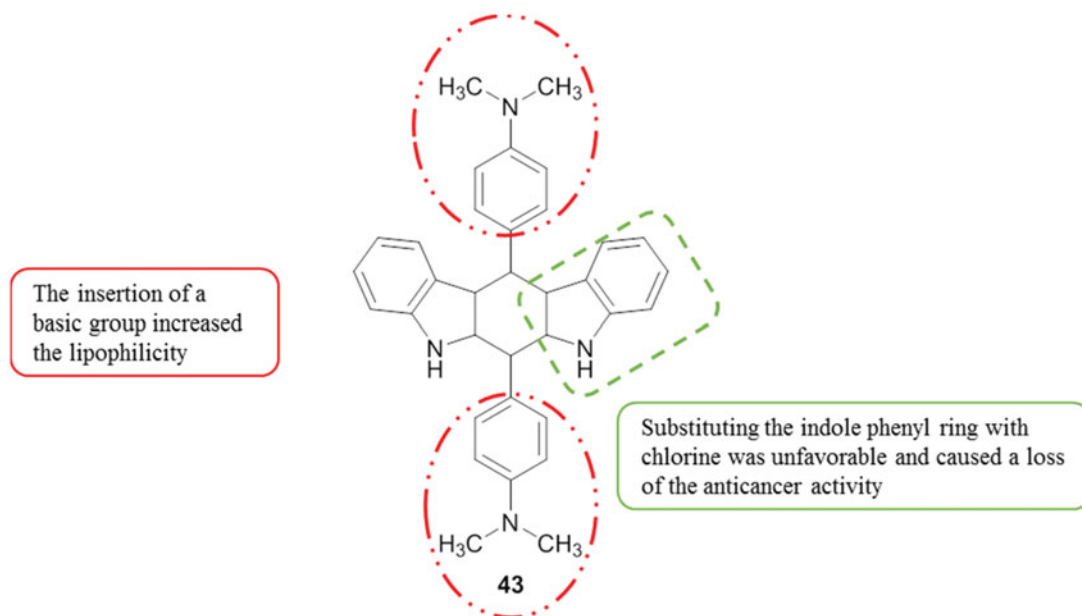
PARP-1 inhibitors. They were also tested in a cell-based assay that evaluated their ability to attenuate the depletion of  $\text{NAD}^+$  levels following hydrogen peroxide insult in PC12 (rat pheochromocytoma) cells. Results showed that two analogues, compounds **42a** and **42b** (Figure 51), displayed potent enzyme activity with  $\text{IC}_{50}$  values of 18 and 25 nM, respectively, as well as high cell permeability (100%  $\text{NAD}^+$  recovery at  $30 \mu\text{M}$ )<sup>84</sup>.



**Figure 50.** Chemical structures of the 1,4-thiazepan-3-ones fused with carbazoles **41a–f**.



**Figure 51.** Chemical structures of 3-substituted-pyrrolocarbazole analogs **42a** and **42b**.

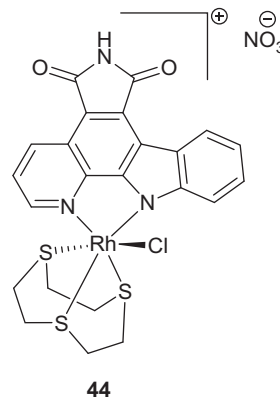


**Figure 52.** Chemical structure of tetrahydroindolocarbazole **43**.

## 4.2. Pentacyclic fused carbazoles containing a five-membered ring and a six-membered ring

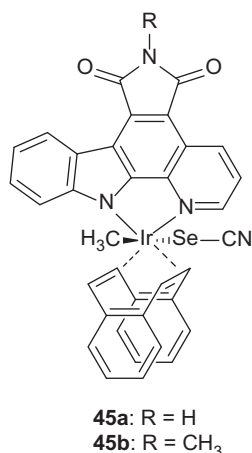
### 4.2.1. Tetrahydroindolo[2,3-*b*]carbazoles

Tetrahydroindolo[2,3-*b*]carbazoles were synthesized to undergo a one dose screening at  $10^{-5} \text{M}$ , followed by a five dose screening for the best compounds using the NCI 60 cell lines list. Compound

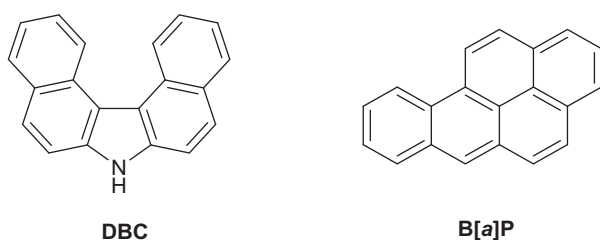


**Figure 53.** Chemical structure of pyridocarbazole-rhodium complex **44**.

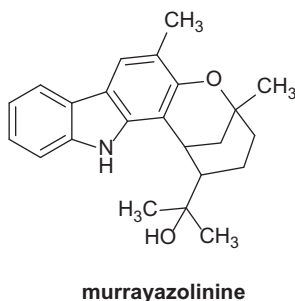




**Figure 54.** Chemical structures of iridium complex **45a** and its N-methylated derivative **45b**.



**Figure 55.** Chemical structure of DBC and B[a]P.



**Figure 56.** Chemical structure of murrayazolinine.

**43** (Figure 52) exhibited the highest anti-cancer activity with growth inhibition at lowest mean value of 21.63%, and GI<sub>50</sub> values ranging from 1.07 to 9.56  $\mu$ M against the tested cell lines<sup>85</sup>.

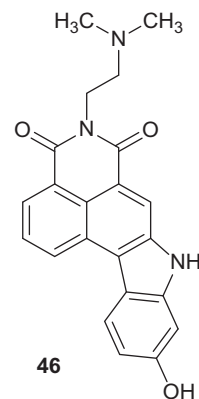
Several inert metal complexes such as pyridocarbazole-rhodium (III) were synthesized and characterized by X-ray crystallography. Stability studies were carried out including evaluation of Pim-1 kinase inhibitory activity. Compound **44** (Figure 53) was found to be a stable rhodium (III) complex and extremely potent inhibitor of Pim-1 kinase (IC<sub>50</sub> around 160 pM)<sup>86</sup>.

The iridium-pyridocarbazole complexes **45a** and **45b** (Figure 54) are highly photocytotoxic compounds. Their anti-angiogenic properties were investigated in a 3D angiogenesis assay. It resulted that **45a** and **45b** are light-independent potent anti-angiogenic agents, very active on the vascular endothelial growth factor<sup>87</sup>.

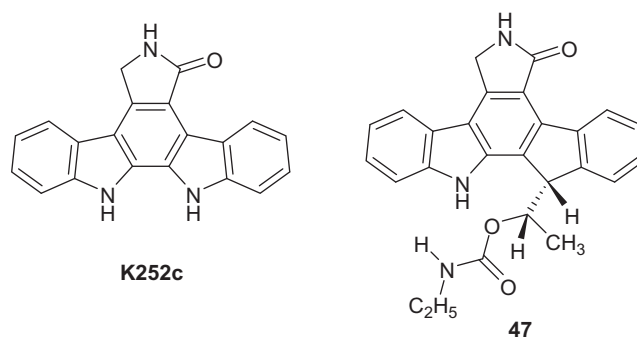
### 4.3. Pentacyclic carbazoles containing two six-membered rings

#### 4.3.1. 7H-Dibenzo[c,g]carbazole

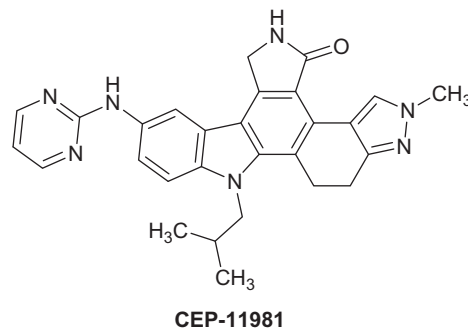
7H-Dibenzo[c,g]carbazole (DBC), benzo[a]pyrene (B[a]P) (Figure 55) and several binary mixtures of both compounds were assessed.



**Figure 57.** Chemical structure of carbazole-amonafide structural hybrid lead candidate **46**.



**Figure 58.** Chemical structures of K252c the indenopyrrolo[3,2-c]carbazole, compound **47**.



**Figure 59.** Chemical structure of CEP-11981.

The biological activity of the binary mixtures was investigated in the HepG2 and WB-F344 (liver cancer) cell lines and the Chinese hamster V79 cell line. These compounds showed an important biological activity on human carcinogens acting on a micro cellular level by modifying cytochrome CYP1A1 expression<sup>88</sup>.

#### 4.3.2. Murrayazolinine

Murrayazolinine (Figure 56) was isolated from *M. euchrestifolia* (Rutaceae) and was evaluated against HL-60 cell line. Murrayazolinine displayed a significant interaction with the caspase-9/caspase-3 pathway, leading to the cellular apoptosis<sup>40</sup>.

#### 4.3.3. Carbazole-amonafide structural hybrids

Preliminary anti-proliferative assays revealed that structural hybrids [4,5-bc]carbazole-amonafide derivatives possessed a good cytotoxic activity with IC<sub>50</sub> values in the sub-micromolar to micromolar range against HTC116 cell line, and were also selective for cancer

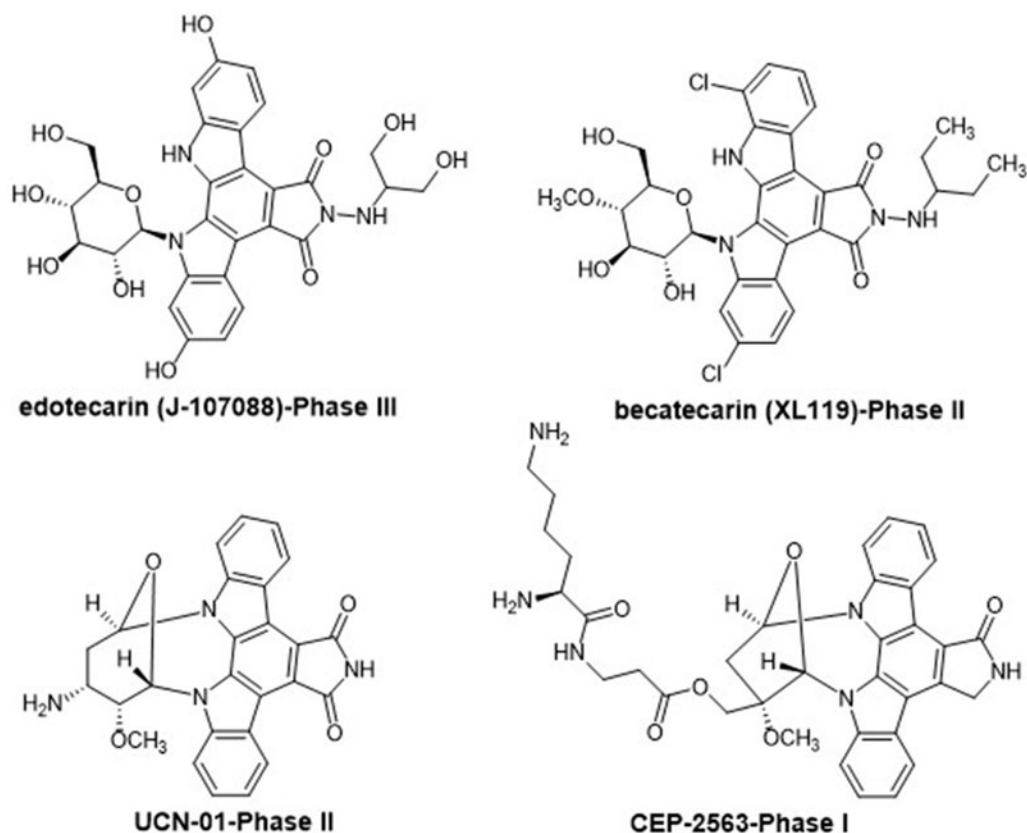


Figure 60. Chemical structures of the synthetic indolocarbazoles in clinical trials.

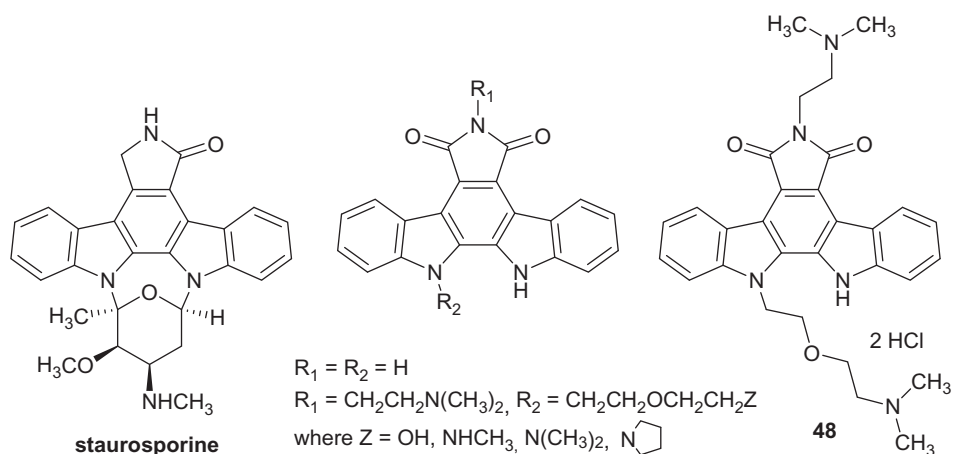


Figure 61. Chemical structures of staurosporine, indolocarbazole analogs and compound 48.

cells when compared to a HEK293 (non-cancerous human embryonic kidney) cell line. Compound **46** (Figure 57) was the lead candidate with an  $IC_{50}$  value of  $0.8 \mu M$  against HTC116 cell line and an  $IC_{50}$  value above  $40 \mu M$  against normal cells<sup>89</sup>.

## 5. Hexacyclic fused carbazoles

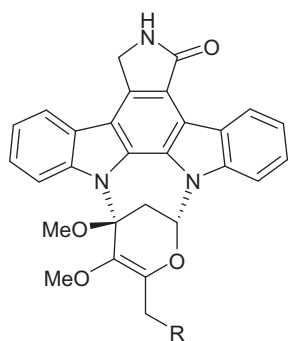
### 5.1. Indenopyrrolocarbazoles

A strong lead candidate, compound **47** (Figure 58), was synthesized from staurosporine aglycone (K252c)<sup>90</sup>. The structure–activity relationship showed that compound **47** is a powerful

tropomyosine kinase TrkA inhibitor; therefore, it was selected as a proof of concept for in vitro and in vivo studies<sup>53</sup>.

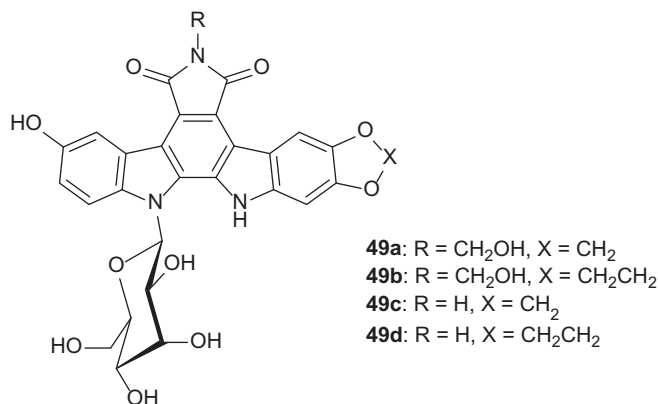
### 5.2. Indazolopyrrolocarbazoles

11-(2-Methylpropyl)-12,13-dihydro-2-methyl-8-(pyrimidin-2-ylamino)-4H-indazolo[5,4-a]pyrrolo[3,4-c]carbazol-4-one (CEP-11981) (Figure 59) is a potent orally active inhibitor of multiple tyrosine kinase receptors (e.g. tyrosine kinase with immunoglobulin and EGF homology domains (TIE2), vascular endothelial growth factor receptor (VEGFR 1–3) and fibroblast growth factor receptor,

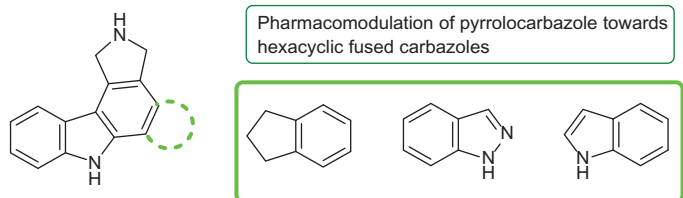


**streptocarbazole A:** R = OH  
**streptocarbazole B:** R = H

**Figure 62.** Chemical structures of streptocarbazoles A and B.



**Figure 63.** Chemical structures of new indolopyrrolocarbazoles **49a–d**.

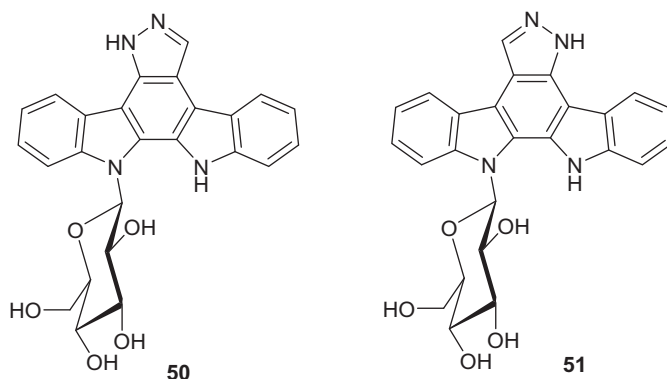


**Figure 64.** From pyrrolocarbazole, a growing approach to design new hexacyclic fused carbazoles as anti-cancer drugs.

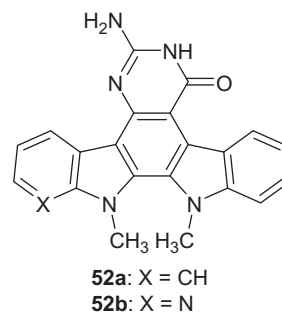
FGFR1), which are potent targets for tumour angiogenesis and vascular maintenance. Furthermore, CEP-11981 exhibits excellent permeability, metabolic stability and pharmacokinetic properties. It was advanced into full development and was in clinical phase I study<sup>91</sup>.

### 5.3. Indolopyrrolocarbazoles

Among the carbazole derivatives, four synthetic indolopyrrolocarbazoles (Figure 60) are currently in clinical trials for cancer therapy. CEP-2563 is active against MTC (medullary thyroid carcinoma) and blocked tyrosine kinase receptors such as Trk family and the platelet-derived growth factor (PDGF) receptor tyrosine kinase<sup>92</sup>. Edotecarin (J-107088) and becatecarin (XL119) which both could intercalate into DNA and edotecarin could additionally stabilize the DNA-topoisomerase I complex. Edotecarin (J-107088) is currently in phase III trials (Pfizer) and becatecarin (XL119) (NCI) is in



**Figure 65.** Chemical structures of indolocarbazoles **50** and **51**.



**Figure 66.** Chemical structures of indolopyrimidocarbazole **52a** and azaindolopyrimidocarbazole **52b**.

clinical trials (phase II) and represent promising approaches for the cancer therapy. UCN-01 is a protein kinase C (PKC) inhibitor and is currently in Phase II trials (NCI) for its activity against pancreatic, lymphoma and breast cancers<sup>92</sup>.

#### 5.3.1. Staurosporine and analogues

A series of indolocarbazoles and staurosporine analogues (Figure 61) were synthesized and tested as anti-proliferative agents against HUVEC (Human Umbilical Vein Endothelial Cells), LoVo (colorectal adenocarcinoma), DLD-1 (colorectal adenocarcinoma) and ST-486 (Burkitt's lymphoma) cell lines. Their anti-angiogenesis activity was also investigated by capillary tube formation in 3-D matrigel matrix. Acero et al.<sup>93</sup> observed on all cell lines that the dimethylaminoalkyl chain in R<sub>1</sub> (Figure 61) enhances both activity and selectivity. Analog **48** (Figure 61), with an IC<sub>50</sub> of 0.1 μM against HUVEC, was one of the most active compounds and the most selective one. The in vivo anti-angiogenic assay using the Lewis lung mice carcinoma model revealed that no tumour reduction was observed, although a slight reduction in metastasis number was noticed<sup>93,94</sup>.

#### 5.3.2. Streptocarbazoles A and B

Isolated from the marine-derived actinomycetes strain *Streptomyces* sp. FMA, streptocarbazoles A and B (Figure 62) were tested as anti-tumoural agents. Streptocarbazole A was cytotoxic against HL-60, A-549 (lung carcinoma), P388 (leukaemia) and HeLa cell lines, with IC<sub>50</sub> values of 1.4, 5.0, 18.9 and 34.5 μM, respectively, and could arrest the cell cycle of HeLa cells at the G2/M phase (at a concentration of 10 μM). Streptocarbazole B was only

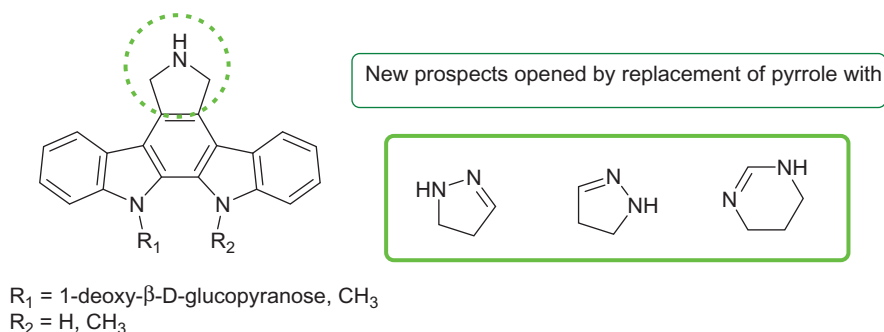


Figure 67. Pharmacomodulation works by replacement of pyrrole moiety.

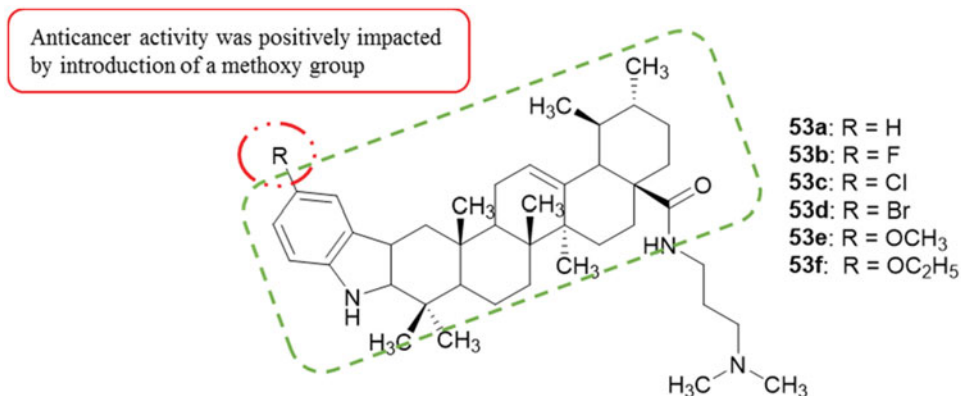


Figure 68. Chemical structures of carbazole derivatives of ursolic acid 53a–f.

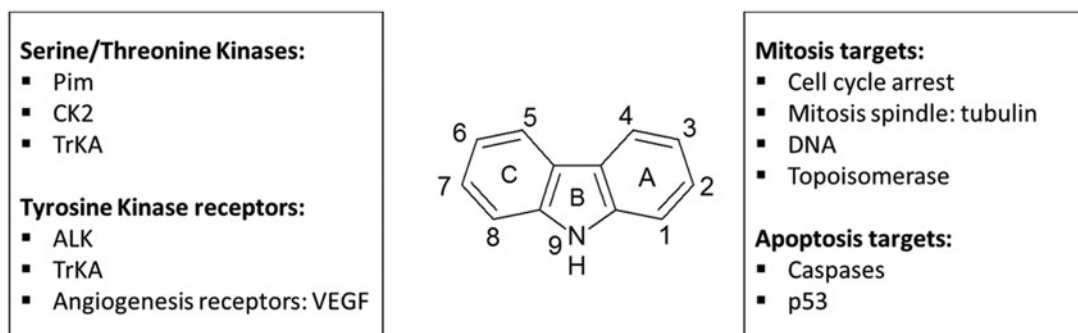


Figure 69. Major anti-tumor activities of carbazole derivatives.

active against P388 and HeLa cells, demonstrating IC<sub>50</sub> values of 12.8 and 22.5 μM, respectively<sup>95</sup>.

physicochemical properties such as hydrosolubility and then to facilitate in vivo investigation.

### 5.3.3. Methylenedioxy- and ethylenedioxy-fused indolopyrrolocarbazoles

The biological activity of indolo[2,3-*a*]carbazole derivatives (Figure 63) was determined as potential anti-cancer agents. Among the analogues, compounds **49a–d** were the most potent compounds against human topoisomerase I and exhibited inhibitory activities with IC<sub>50</sub> values in the micromolar range (from 3.2 to 5.4 μM)<sup>96</sup>.

In summary, the scaffold pyrrolocarbazole was extensively used as a part of hexacyclic fused carbazoles. Three sub-series of related compounds were developed, namely indeno-, indazo- and indolo-pyrrolocarbazoles (Figure 64). For some the additional presence of a sugar moiety is also to notice (e.g. edotecarin, compounds **49a–d**). It is an important point to modulate their

### 5.4. Indolopyrazolocarbazoles

Glycosylated indolopyrazolocarbazole analogues of K252c (Figure 58) have been synthesized to improve their cellular potencies leading to two compounds: 13-(1-deoxy-β-D-glucopyranos-1-yl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazole **50** and 12-(1-deoxy-β-D-glucopyranos-1-yl)-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrazolo[3,4-*c*]carbazole **51** (Figure 65)<sup>97</sup>. Interestingly, compounds **50** and **51** were active toward HCT116 (human colon carcinoma) cell line with similar IC<sub>50</sub> values of 12 and 11 μM, respectively.

### 5.5. Indolopyrimidocarbazoles and related

A series of indolo[2,3-*a*]pyrimido[5,4-*c*]carbazoles and azaindolopyrimidocarbazoles was synthesized and their anti-cancer activity

was evaluated through topoisomerase II inhibition and in cellulo assay using the NCI-60 cell line. Although no topoisomerase II inhibition was observed, compound **52a** (Figure 66) was found to inhibit in vitro the growth of HCT-15 (colon carcinoma), SK-MEL-2 (melanoma), and ACHN, CAKI-1 and UO-31 (renal adenocarcinoma) cell lines with  $GI_{50}$  values in the low micromolar range. The less toxic azaindolocarbazole **52b** (Figure 66) also showed cytostatic activity against NCI-H522 (non-small-cell lung cancer) and UO-31 cell lines<sup>98</sup>.

In the particular point of the replacement of the pyrrolo moiety of indolopyrrolocarbazole either by a pyrazolo or by a pyrimido ring systems (Figure 67), new active hexacyclic derivatives demonstrated cytotoxic activity on colon carcinoma cell lines (e.g. HCT116, HCT-15).

## 6. Heptacyclic fused carbazoles

### 6.1. Carbazole derivatives of ursolic acid

A series of carbazole derivatives of ursolic acid was synthesized and assayed against two human liver cancer cell lines (SMMC-7721 and HepG2) using the MTT colorimetric method. From the results, compounds **53a–f** (Figure 68) displayed pronounced cytotoxic activities with  $IC_{50}$  values below  $10 \mu M$ . Compound **53e** was found to be the most active compound with  $IC_{50}$  values of  $1.08 \pm 0.22$  and  $1.26 \pm 0.17 \mu M$  against SMMC-7721 and HepG2 cells, respectively, comparable to those of doxorubicin. In addition, **53e** showed reduced cytotoxicity against noncancerous  $LO_2$  cells with an  $IC_{50}$  value of  $5.75 \pm 0.48 \mu M$ <sup>99</sup>.

## 7. Conclusion

Cancer is a very complex disease and the increase of the biological targets can be synergistically coordinated to relieve patients from cancer burden. Many new cancer therapies have been developed in the last years, but this research field still presents many challenges. Among the natural products, the carbazole alkaloids have shown several biological activities (Figure 69). Since 2012, we presented the major anti-tumoural activities of natural and synthetic carbazole derivatives.

In a recent study, Iman et al.<sup>100</sup> showed that the combination of many mechanisms of action were observed in the case of girinimbine which resulted in an induction of G0/G1 phase arrest, an upregulation of two cyclin-dependent kinase proteins p21 and p27, an activation of caspase-3 and caspase-9, downregulation of Bcl-2 and upregulation of Bax in girinimbine-treated cells. Another activity was seen on the upregulation of p53. Induction of apoptosis by girinimbine was also investigated in vivo by using zebrafish embryos, with results demonstrating significant distribution of apoptotic cells in embryos after a 24-h treatment period<sup>100</sup>. Some compounds are currently following clinical trial phases and the optimal structure has not yet been found. Carbazole derivatives have been recently described by Diaz et al. for their anti-tumour activity with the microtubule targeting and could inhibit tubulin assembly<sup>101</sup>. Many potential compounds can be the future candidate for the cancer chemotherapy, with the purpose of a multi-target therapy.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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## References

- WHO methods and data sources for global burden of disease estimates 2000–2015, Department of information, evidence and research WHO, Geneva, January 2017, available at [http://www.who.int/healthinfo/global\\_burden\\_disease/GlobalDALYmethods\\_2000\\_2015.pdf](http://www.who.int/healthinfo/global_burden_disease/GlobalDALYmethods_2000_2015.pdf) [last accessed 26 Jul 2018].
- Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015;136:E359–86.
- Schmidt AW, Reddy KR, Knölker H-J. Occurrence, biogenesis, and synthesis of biologically active carbazole alkaloids. *Chem Rev* 2012;112:3193–328.
- Knölker H-J, Reddy KR. Isolation and synthesis of biologically active carbazole alkaloids. *Chem Rev* 2002;102:4303–427.
- Graebe C, Glazer C. First isolation of carbazole from coal tar. *Ber Dtsch Chem Ges* 1872;5:12.
- Chakraborty DP, Roy S, Carbazole alkaloids IV. In: *Fortschritte der Chemie organischer Naturstoffe/Progress in the Chemistry of Organic Natural Products*. Wien GmbH: Springer-Verlag; 2003; vol. 85:125–230.
- Bashir M, Bano A, Ijaz AS, Chaudhary BA. Recent developments and biological activities of N-substituted carbazole derivatives: a review. *Molecules* 2015;20:13496–517.
- Bajaj YPS. Ochrosia sp.: In vitro production of ellipticine an antitumor agent. Chapter III.7. In: Chénieux C, Ramawat KG, Rideau M, eds. *Medicinal and aromatic plants I. Biotechnology in agriculture and forestry*. vol. 4. Berlin Heidelberg: Springer-Verlag; 1988.
- (a) Sinicropi MS, Iacopetta D, Rosano C, et. al. N-thioalkyl-carbazoles derivatives as new anti-proliferative agents: synthesis, characterization, and molecular mechanism evaluation. *J Enzyme Inhib Med Chem* 2018;33:434–44. (b) Jiang H, Zhang WJ, Li PH, et.al. Synthesis and biological evaluation of novel carbazole-rhodanine conjugates as topoisomerase II inhibitors. *Bioorg Med Chem Lett* 2018;28:



- 1320–3. (c) Saturnino C, Caruso A, Iacopetta D, et al. Inhibition of human topoisomerase II by new N,N,N-trimethylethanammonium iodide alkylcarbazole derivatives. *ChemMedChem* 2018;13:2635–43.
10. Elliptinium acetate, NCI Drug Dictionary, available at <https://www.cancer.gov/publications/dictionaries/cancer-drug/def/elliptinium-acetate> [last accessed 26 Apr 2018].
11. Alectinib approved for (ALK) positive metastatic non-small cell lung cancer (NSCLC), U.S. FDA, Approved Drugs, available at <https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm584082.htm> [last accessed 26 Apr 2018].
12. Alecensa, alectinib, product information, EMA, Human medicines, available at [http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/004164/human\\_med\\_002068.jsp&mid=WC0b01ac058001d124](http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/004164/human_med_002068.jsp&mid=WC0b01ac058001d124) [last accessed 26 Jul 2018].
13. Ruiz-Ceja KA, Chirino YI. Current FDA-approved treatments for non-small cell lung cancer and potential biomarkers for its detection. *Biomed Pharmacother* 2017;90:24–37.
14. Midostaurin approved for the treatment of adult patients with newly diagnosed acute myeloid leukemia (AML), U.S. FDA, Approved Drugs, available at <https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm555756.htm> [last accessed 26 Apr 2018].
15. Rydapt, midostaurin, product information, EMA, Human medicines, available at [http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/004095/human\\_med\\_002155.jsp&mid=WC0b01ac058001d124](http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/004095/human_med_002155.jsp&mid=WC0b01ac058001d124) [last accessed 26 Jul 2018].
16. Stone RM, Manley PW, Larson RA, Capdeville R. Midostaurin: its odyssey from discovery to approval for treating acute myeloid leukemia and advanced systemic mastocytosis. *Blood Adv* 2018;2:444–53.
17. Gutierrez L, Jang M, Zhang T, et al. Midostaurin reduces regulatory T cells markers in acute myeloid leukemia. *Sci Rep* 2018;8:17544.
18. (a) Janosik T, Rannug A, Rannug U, et al. Chemistry and properties of indolocarbazoles. *Chem Rev* 2018;118:9058–128. (b) Tsutsumi LS, Gündisch D, Sun D. Carbazole scaffold in medicinal chemistry and natural products: a review from 2010–2015. *Curr Top Med Chem* 2016;16:1290–313. (c) Shaikh MS, Karpoomath R, Thapliyal N, et al. Current perspective of natural alkaloid carbazole and its derivatives as antitumor agents. *Anticancer Agents Med Chem* 2015;15:1049–65.
19. Council of Scientific & Industrial Research (IN). Carbazole-bonded pyrrolo[2,1-c][1,4]benzodiazepine hybrids as potential anticancer agents and process for their preparation. US 8,927,538. 2015.
20. Bristol-Meyers Squibb Co. Carbazole and carboline kinase inhibitors. US 0,058,988. 2012.
21. Philip Morris Products SA. Carbazole and carboline derivatives, and preparation and therapeutic applications thereof. US 0,274,258. 2013.
22. University of Kansas. Formulations of indole-3-carbinol derived antitumor agents with increased oral bioavailability. US 0,184,590. 2012.
23. CNRS. Use of carbazole phenone derivatives for the treatment of cancer. FR 0,051,491. 2012.
24. Bristol-Meyers Squibb Co. Carbazole compounds useful as bromodomain inhibitors. EP 2,961,748. 2018.
25. Health Research Inc. Compounds and methods for treating cancers. US 0,024,083. 2016.
26. Incuron LCC. Carbazole compounds and therapeutic uses of the compounds. US 9,108,916. 2015.
27. Novogen Ltd. Functionalized and substituted carbazoles as anti-cancer agents. US 0,158,636. 2017.
28. GTx Inc. Selective androgen receptor degrader (SARD) ligands and methods of use thereof. US 0,166,526. 2017.
29. Mitra K, Basu U, Khan I, et al. Remarkable anticancer activity of ferrocenyl-terpyridine platinum (II) complexes in visible light with low dark toxicity. *Dalton Trans* 2014;43:751–63.
30. Kolli SK, Prasad B, Babu PV, et al. TFAA/H<sub>3</sub>PO<sub>4</sub> mediated unprecedented N-acylation of carbazoles leading to small molecules possessing anti-proliferative activities against cancer cells. *Org Biomol Chem* 2014;12:6080–4.
31. Shrugesh Kumar TO, Mahadevan KM, Kumara MN. Synthesis and cytotoxic studies of 2,3-dimethylindoles and tetrahydrocarbazoles. *Int J Pharm Pharm Sci* 2014;6:137–40.
32. Bandgar BP, Adsul LK, Chavan HV, et al. Synthesis, biological evaluation, and molecular docking of N-{3-[3-(9-methyl-9H-carbazol-3-yl)-acryloyl]-phenyl}-benzamide/amide derivatives as xanthine oxidase and tyrosinase inhibitors. *Bioorg Med Chem* 2012;20:5649–57.
33. Chou YS, Chang CC, Chang TC, et al. Photo-induced antitumor effect of 3,6-bis(1-methyl-4-vinylpyridinium)carbazole diiodide. *Biomed Res Int* 2013;2013:1.
34. Głuszyńska A. Biological potential of carbazole derivatives. *Eur J Med Chem* 2015;94:405–26.
35. Liao LJ, Kang CC, Jan IS, et al. Improved diagnostic accuracy of malignant neck lumps by a simple BMVC staining assay. *Analyst* 2009;134:708–11.
36. Gu Y, Lin D, Tang Y, et al. A light-up probe targeting for Bcl-2 2345 G-quadruplex DNA with carbazole TO. *Spectrochim Acta A Mol Biomol Spectrosc* 2018;191:180–8.
37. Francisco CS, Rodrigues LR, Cerqueira NM, et al. Novel benzoporsalen analogues: synthesis, biological activity and molecular docking studies. *Eur J Med Chem* 2014;87:298–305.
38. Yoon S, Kim J-H, Lee YJ, et al. A novel carbazole derivative, MHY407, sensitizes cancer cells to doxorubicin-, etoposide-, and radiation treatment via DNA damage. *Eur J Pharmacol* 2012;697:24–31.
39. Chen LC, Juang SH, Chang KM, et al. Synthesis and antiproliferative evaluation of amide-containing anthraquinone, xanthone, and carbazole. *Chem Pharm Bull (Tokyo)* 2014;62:106–11.
40. Ito C, Itoigawa M, Nakao K, et al. Apoptosis of HL-60 leukemia cells induced by carbazole alkaloids isolated from *Murraya euchrestifolia*. *J Nat Med* 2012;66:357–61.
41. Vega FM, Ridley AJ. Rho GTPases in cancer cell biology. *FEBS Lett* 2008;582:2093–101.
42. Lin W, Wang Y, Lin S, et al. Induction of cell cycle arrest by the carbazole alkaloid Clauszoline-I from *Clausena vestita* DD Tao via inhibition of the PKC $\delta$  phosphorylation. *Eur J Med Chem* 2012;47:214–20.
43. Saturnino C, Palladino C, Napoli M, et al. Synthesis and biological evaluation of new N-alkylcarbazole derivatives as STAT3 inhibitors: preliminary study. *Eur J Med Chem* 2013;60:112–9.
44. Peng WW, Zeng GZ, Song WW, Tan NH. A new cytotoxic carbazole alkaloid and two new other alkaloids from *Clausena excavata*. *Chem Biodivers* 2013;10:1317–21.



45. Maneerat W, Ritthiwigrom T, Cheenpracha S, et al. Bioactive carbazole alkaloids from *Clausena wallichii* roots. *J Nat Prod* 2012;75:741–6.
46. Çiftçi GA, Temel HE, Yıldırım ŞU, et al. Apoptotic effects of some carbazole derivatives on lung carcinoma and glioma cell lines. *Med Chem Res* 2013;22:3751–9.
47. Vlaar CP, Castillo-Pichardo L, Medina JI, et al. Design, synthesis and biological evaluation of new carbazole derivatives as anti-cancer and anti-migratory agents. *Bioorg Med Chem* 2018;26:884–90.
48. Kalathiya U, Padariya M, Baginski M. Molecular modeling and evaluation of novel dibenzopyrrole derivatives as telomerase inhibitors and potential drug for cancer therapy. *IEEE/ACM Trans Comput Biol Bioinforma TCBB* 2014;11:1196–207.
49. Sun L, Wu Y, Liu Y, et al. Novel carbazole sulfonamide derivatives of antitumor agent: synthesis, antiproliferative activity and aqueous solubility. *Bioorg Med Chem Lett* 2017;27:261–5.
50. Niu F, Liu Y, Jing Z, et al. Novel carbazole sulfonamide microtubule-destabilizing agents exert potent antitumor activity against esophageal squamous cell carcinoma. *Cancer Lett* 2018;420:60–71.
51. Panno A, Sinicropi MS, Caruso A, et al. New trimethoxybenzamides and trimethoxyphenylureas derived from dimethylcarbazole as cytotoxic agents. Part I. *J Heterocycl Chem* 2014;51:294–302.
52. Caruso A, Sinicropi MS, Lancelot J-C, et al. Synthesis and evaluation of cytotoxic activities of new guanidines derived from carbazoles. *Bioorg Med Chem Lett* 2014;24:467–72.
53. Rongved P, Kirsch G, Bouaziz Z, et al. Indenoindoles and cyclopentacarbazoles as bioactive compounds: synthesis and biological applications. *Eur J Med Chem* 2013;69:465–79.
54. Maneerat W, Ritthiwigrom T, Cheenpracha S, Laphookhieo S. Carbazole alkaloids and coumarins from *Clausena lansium* roots. *Phytochem Lett* 2012;5:26–8.
55. Santio NM, Vahakoski RL, Rainio E-M, et al. Pim-selective inhibitor DHPCC-9 reveals Pim kinases as potent stimulators of cancer cell migration and invasion. *Mol Cancer* 2010;9:279.
56. Akué-Gédu R, Letribot B, Saugues E, et al. Kinase inhibitory potencies and in vitro antiproliferative activities of N-10 substituted pyrrolo[2,3-a]carbazole derivatives. *Bioorg Med Chem Lett* 2012;22:3807–9.
57. Letribot B, Akué-Gédu R, Santio NM, et al. Use of copper(I) catalyzed azide alkyne cycloaddition (CuAAC) for the preparation of conjugated pyrrolo[2,3-a]carbazole Pim kinase inhibitors. *Eur J Med Chem* 2012;50:304–10.
58. Moreau P, Dezhenkova LG, Anizon F, et al. New potent and selective inhibitor of Pim-1/3 protein kinases sensitizes human colon carcinoma cells to doxorubicin. *Anticancer Agents Med Chem* 2014;14:1228–36.
59. Giraud F, Bourhis M, Nauton L, et al. New N-1,N-10-bridged pyrrolo[2,3-a]carbazole-3-carbaldehydes: synthesis and biological activities. *Bioorg Chem* 2014;57:108–15.
60. Giraud F, Akué-Gédu R, Nauton L, et al. Synthesis and biological activities of 4-substituted pyrrolo [2,3-a]carbazole Pim kinase inhibitors. *Eur J Med Chem* 2012;56:225–36.
61. Ichikawa S, Tatebayashi N, Matsuda A. Synthesis of C-glycosyl pyrrolo[3,4-c]carbazole-1,3(2H,6H)-diones as a scaffold for check point kinase 1 inhibitors. *J Org Chem* 2013;78:12065–75.
62. Suchaud V, Gavara L, Saugues E, et al. Identification of 1,6-dihydropyrzolo[4,3-c]carbazoles and 3,6-dihydropyrzolo[3,4-c]carbazoles as new Pim kinase inhibitors. *Bioorg Med Chem* 2013;21:4102–11.
63. Vairavelu L, Zeller M, Prasad KR. Solvent-free synthesis of heteroannulated carbazoles: a novel class of anti-tumor agents. *Bioorg Chem* 2014;54:12–20.
64. Song Z, Wang M, Zhang A. Alectinib: a novel second generation anaplastic lymphoma kinase (ALK) inhibitor for overcoming clinically-acquired resistance. *Acta Pharm Sin B* 2015;5:34–7.
65. Kinoshita K, Asoh K, Furuichi N, et al. Design and synthesis of a highly selective, orally active and potent anaplastic lymphoma kinase inhibitor (CH5424802). *Bioorg Med Chem* 2012;20:1271–80.
66. Kodama T, Tsukaguchi T, Yoshida M, et al. Selective ALK inhibitor alectinib with potent antitumor activity in models of crizotinib resistance. *Cancer Lett* 2014;351:215–21.
67. Ravichandiran P, Jegan A, Premnath D, et al. Synthesis, molecular docking and cytotoxicity evaluation of novel 2-(4-amino-benzosulfonyl)-5H-benzo[b]carbazole-6,11-dione derivatives as histone deacetylase (HDAC8) inhibitors. *Bioorg Chem* 2014;53:24–36.
68. Kok YY, Mooi LY, Ahmad K, et al. Anti-tumor promoting activity and antioxidant properties of girinimbine isolated from the stem bark of *Murraya koenigii* S. *Molecules* 2012;17:4651–60.
69. Padmaja P, Rao GK, Indrasena A, et al. Synthesis and biological evaluation of novel pyrano[3,2-c]carbazole derivatives as anti-tumor agents inducing apoptosis via tubulin polymerization inhibition. *Org Biomol Chem* 2015;13:1404–14.
70. Uvarani C, Jaivel N, Sankaran M, et al. Axially chiral biscarbazoles and biological evaluation of the constituents from *Murraya koenigii*. *Fitoterapia* 2014;94:10–20.
71. Das R, Bhattacharya K, Sarkar S, et al. Mahanine synergistically enhances cytotoxicity of 5-fluorouracil through ROS-mediated activation of PTEN and p53/p73 in colon carcinoma. *Apoptosis Int J Program Cell Death* 2014;19:149–64.
72. Das R, Bhattacharya K, Samanta SK, et al. Improved chemosensitivity in cervical cancer to cisplatin: synergistic activity of mahanine through STAT3 inhibition. *Cancer Lett* 2014;351:81–90.
73. Prudent R, Moucadel V, Nguyen C-H, et al. Antitumor activity of pyridocarbazole and benzopyridoindole derivatives that inhibit protein kinase CK2. *Cancer Res* 2010;70:9865–74.
74. Mori R, Kato A, Komenoi K, et al. Synthesis and in vitro antitumor activity of novel 2-alkyl-5-methoxycarbonyl-11-methyl-6H-pyrido[4,3-b]carbazol-2-ium and 2-alkylellipticin-2-ium chloride derivatives. *Eur J Med Chem* 2014;82:16–35.
75. Sharma M, Agarwal A, Kishore D, Paliwal S. Quantitative structure activity relationship (QSAR) analysis of olivacine derivatives as topoisomerase inhibitors. *Int J Pharm Pharm Sci* 2012;4:198–204.
76. Indumathi T, Muthusankar A, Shanmughavel P, Prasad KR. Synthesis of hetero annulated carbazoles: exploration of in vitro cytotoxicity and molecular docking studies. *MedChemComm* 2013;4:450.
77. Indumathi T, Prasad KR. Multicomponent synthesis of pyrrolo[2,3-a]carbazoles and cytotoxic activity. *Med Chem Res* 2014;23:4345–55.

78. Li B, Yue Z-Z, Feng J-M, et al. Design and synthesis of pyrido[3,2- $\alpha$ ]carbazole derivatives and their analogues as potent antitumor agents. *Eur J Med Chem* 2013;66:531–9.
79. Hamilton PL, Arya DP. Natural product DNA major groove binders. *Nat Prod Rep* 2012;29:134–43.
80. Murali K, Sparkes HA, Rajendra Prasad KJ. Synthesis of hetero annulated isoxazolo-, pyrido- and pyrimido carbazoles: screened for in vitro antitumor activity and structure activity relationships, a novel 2-amino-4-(3'-bromo-4'-methoxyphenyl)-8-chloro-11H-pyrimido[4,5-a]carbazole as an antitumor agent. *Eur J Med Chem* 2017;128:319–31.
81. (a) Issa S, Walchshofer N, Kassab I, et al. Synthesis and antiproliferative activity of oxazinocarbazole and N,N-bis(carbazolylmethyl)amine derivatives. *Eur J Med Chem* 2010; 45:4: 2567–77. (b) Bouaziz Z, Issa S, Gentili J, et al. Biologically active carbazole derivatives: focus on oxazinocarbazoles and related compounds. *J Enzyme Inhib Med Chem* 2015; 30:180–8.
82. Naik PN, Khan A, Kusurkar RS. Intramolecular Diels–Alder reaction for the synthesis of tetracyclic carbazoles and isocanthines. *Tetrahedron* 2013;69:10733–8.
83. Shi F, Zeng X-N, Cao X-D, et al. Design and diversity-oriented synthesis of novel 1, 4-thiazepan-3-ones fused with bioactive heterocyclic skeletons and evaluation of their antioxidant and cytotoxic activities. *Bioorg Med Chem Lett* 2012;22:743–6.
84. Dunn D, Husten J, Ator MA, Chatterjee S. Novel poly (ADP-ribose) polymerase-1 inhibitors. *Bioorg Med Chem Lett* 2007;17:542–5.
85. El Sayed MT, Ahmed KM, Mahmoud K, Hilgeroth A. Synthesis, cytostatic evaluation and structure activity relationships of novel bis-indolylmethanes and their corresponding tetrahydroindolocarbazoles. *Eur J Med Chem* 2015;90:845–59.
86. Dieckmann S, Riedel R, Harms K, Meggers E. Pyridocarbazole-rhodium (III) complexes as protein kinase inhibitors. *Eur J Inorg Chem* 2012;2012:813–21.
87. Kastl A, Wilbuer A, Merkel AL, et al. Dual anticancer activity in a single compound: visible-light-induced apoptosis by an antiangiogenic iridium complex. *Chem Commun Camb Engl* 2012;48:1863–5.
88. Gábelová A, Poláková V, Procházka G, et al. Sustained induction of cytochrome P4501A1 in human hepatoma cells by co-exposure to benzo[a]pyrene and 7H-dibenzo[c,g]carbazole underlies the synergistic effects on DNA adduct formation. *Toxicol Appl Pharmacol* 2013; 271:1–12.
89. Rozovsky A, Regozin E, Oron-Herman M, et al. Synthesis of antitumor carbazole-amonafide structural hybrids. *Eur J Org Chem* 2015;2015:1811–8.
90. Esvan YJ, Giraud F, Pereira E, et al. Synthesis and biological activity of pyrazole analogues of the staurosporine aglycon K252c. *Bioorg Med Chem* 2016;24:3116–24.
91. Hudkins RL, Becknell NC, Zulli AL, et al. Synthesis and biological profile of the pan-vascular endothelial growth factor receptor/tyrosine kinase with immunoglobulin and epidermal growth factor-like homology domains 2 (VEGF-R/TIE-2) inhibitor 11-(2-methylpropyl)-12,13-dihydro-2-methyl-8-(pyrimidin-2-ylamino)-4H-indazolo[5,4-a]pyrrolo[3,4-c]carbazol-4-one (CEP-11981): a novel oncology therapeutic agent. *J Med Chem* 2012;55:903–13.
92. Palanisamy SK, Rajendran NM, Marino A. Natural products diversity of marine *Ascidians* (Tunicates; Ascidiacea) and successful drugs in clinical development. *Nat Prod Bioprospect* 2017;7:1–111.
93. Acero N, Braña MF, Añorbe L, et al. Synthesis and biological evaluation of novel indolocarbazoles with anti-angiogenic activity. *Eur J Med Chem* 2012;48:108–13.
94. Sherer C, Snape TJ. Heterocyclic scaffolds as promising anticancer agents against tumours of the central nervous system: exploring the scope of indole and carbazole derivatives. *Eur J Med Chem* 2015;97:552–60.
95. Fu P, Yang C, Wang Y, et al. Streptocarbazoles A and B, two novel indolocarbazoles from the marine-derived actinomycete strain *Streptomyces* sp. FMA. *Org Lett* 2012; 14:2422–5.
96. Zembower DE, Xie Y, Koohang A, et al. Methylenedioxy- and ethylenedioxy-fused indolocarbazoles: potent human topoisomerase I inhibitors and antitumor agents. *Anticancer Agents Med Chem* 2012;12:1117–31.
97. Douara B, Esvan YJ, Pereira E, et al. Synthesis and antiproliferative evaluation of glucosylated pyrazole analogs of K252c. *Tetrahedron* 2018;74:892–901.
98. Pierce LT, Cahill MM, Winfield HJ, McCarthy FO. Synthesis and identification of novel indolo[2,3-a]pyrimido[5,4-c]carbazoles as a new class of anti-cancer agents. *Eur J Med Chem* 2012;56:292–300.
99. Gu W, Hao Y, Zhang G, et al. Synthesis, in vitro antimicrobial and cytotoxic activities of new carbazole derivatives of ursolic acid. *Bioorg Med Chem Lett* 2015;25:554–7.
100. Iman V, Mohan S, Abdelwahab SI, et al. Anticancer and anti-inflammatory activities of girinimbine isolated from *Murraya koenigii*. *Drug Des Devel Ther* 2016;Volume11: 103–21.
101. Diaz P, Horne E, Xu C, et al. Modified carbazoles destabilize microtubules and kill glioblastoma multiform cells. *Eur J Med Chem* 2018;159:74–89.